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# Phytopathology

## An International Journal

## Official Organ of
## The American Phytopathological Society

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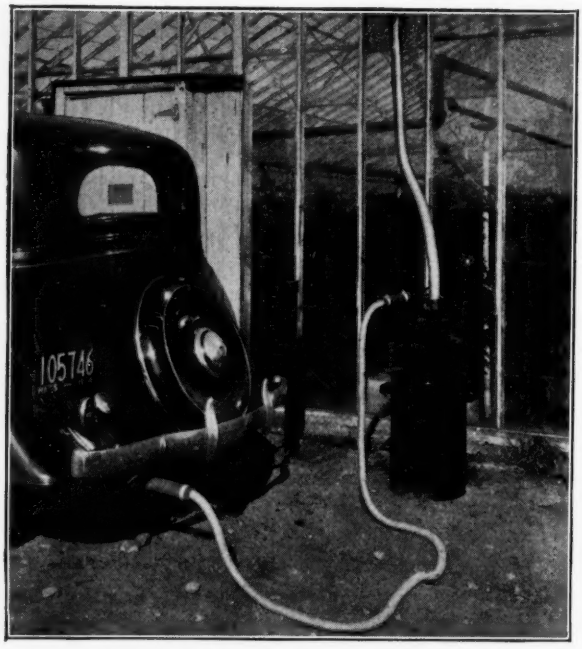


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PHYTOPATHOLOGY

VOLUME 24 NUMBER 9

SEPTEMBER, 1934

# PHYTOPATHOLOGICAL AND TAXONOMIC ASPECTS OF OPHIOBOLUS, PYRENOPHORA, HELMINTHOSPORIUM, AND A NEW GENUS, COCHLIOBOLUS

CHARLES DRECHSLER

(Accepted for publication December 13, 1933)

## OPHIOBOLUS HERPOTRICHUS AND O. GRAMINIS

The genus Ophiobolus, erected by Riess (65) in 1854, was extended into the sphere of phytopathological interest in 1881 with the transfer to it by Saccardo (66) of the fungus described by him (67) only 6 years earlier as *Rhaphidophora graminis* together with the form that, originally described by Fries (26, v. 2, p. 504) as *Sphaeria herpotricha,* later had been cited as *Rhaphidospora herpotricha* (Fries) by Fuckel (28), and as *Rhaphidophora herpotricha* by the Tuslasne brothers (73). The transfer, as is evident from a statement of somewhat later date (68, v. 2, p. 337), was carried out because of nomenclatorial necessity, the genera Rhaphidospora Fries and Rhaphidophora Ces. and De Not., assuming they can be regarded as separate taxonomic entities on strictly formal grounds, having been found homonymous with genera of seed plants erected earlier by Nees and Hasskarl, respectively.

The earlier descriptive literature of the 2 fungi conveys little intimation of pathogenic relationships, the only direct intimation, indeed, here being contained in the somewhat reluctant statement by the Tulasne brothers that they had found *Ophiobolus herpotrichus* on fading wheat culms as early as the end of June, its appearance there being associated with subsequent premature yellowing of the heads and blackening of the stems. In 1880 Cugini (6) found a wheat disease in Italy, associated with blackening and killing of the roots, and with the development of a black incrustation over the surface of the stem. This disease he attributed to *Rhaphidospora herpotricha,* on the strength of an identification by Passerini; and the same fungus, referred to as *O. herpotrichus,* was held responsible for a similar disease encountered by Cugini (7) in 1889. In the meantime Morini (55), in investigating the causes of a new disease of wheat in Italy, had discovered, though apparently only on a single diseased specimen, a fungus that he held to agree well with *O. herpotrichus* except in having shorter asci and

ascospores, these structures measuring only 90–114 μ and 75–84 μ, respectively. This fungus he had described as *O. herpotrichus* var. *breviasci.*

The dimensions given by Morini together with his characterization of the ascospores as pale yellowish make it hard to avoid the conclusion that he was dealing not with *Ophiobolus herpotrichus* at all, but with *O. graminis.* Of the latter species he makes no mention, a fact that, under the circumstances, is difficult to explain except on the assumption that he was unaware of its having been described. For that matter neither of Cugini's papers makes mention of *O. graminis,* though the statement in the later one to the effect that Saccardo had said in the "Sylloge fungorum" that the species involved (presumably *O. herpotrichus*) was found on Cynodon and Agrostis, indicates that Cugini may have inadvertently confused or, perhaps, purposely integrated the portion of Saccardo's diagnosis of *O. graminis,* referring to habitat (in which Cynodon and Agropyron were cited as hosts) with the diagnosis of *O. herpotrichus.* It is possible therefore that Cugini's exclusive preoccupation with *O. herpotrichus* may have been due less to lack of knowledge concerning the other form reported on graminaceous hosts than to doubt as to the independence of that form from the much older and better established species the graminicolous character of which apparently had already become somewhat a matter of accepted tradition.

Such doubt, at all events, would seem to have prevailed after Prillieux and Delacroix (61) in 1890 reported *Ophiobolus graminis* as the cause of the wheat disease in France long known there as *piétin* or *maladie du pied.* For Frank (22), in reporting *O. herpotrichus* as responsible for serious damage to wheat in Germany in 1894, stated not only that the fungus had been known for some time in Italy, but also that it had a few years earlier been observed in the region surrounding Paris, there having occasioned the same injuries as in Germany. Any possible uncertainty as to which French report was alluded to in the statement, was dispelled in two later publications (23; 24, v. 2, p. 306–307) in the second of which, especially, the paper by Prillieux and Delacroix was explicitly cited in connection with the representation that *O. herpotrichus* had been found in France as the cause of *piétin* or *maladie du pied.* As the French authors had clearly set forth that their identification of the parasite causing *piétin* was based on its correspondence to Saccardo's description of *O. graminis,* especially with respect to dimensions of asci and ascospores, and as Frank was evidently cognizant of the different dimensions attributed to *O. herpotrichus,* since he cited them in one of his publications (24), it may be inferred that he regarded the 2 species as one in spite of knowledge of the differences in morphology ascribed to them.

This inclusive species concept became of moment in phytopathological

literature when in Frank's several publications *Ophiobolus herpotrichus* was set forth as one of the most destructive parasites of wheat in Germany, with the imputed destructiveness being, moreover, vividly brought into relief in the formidable term *Weizenhalmtöter* applied to the fungus. Unfortunately, these papers contain few clues as to the extent to which their author may have been dealing with *O. herpotrichus* on the one hand and with *O. graminis* on the other. The most tangible clue undoubtedly is to be found in the colored plate accompanying the last of Frank's (25) papers on the *Weizenhalmtöter,* which it may be presumed was prepared under his direction or must at least have met with his approval. In this plate the figures of the asci and ascospores show proportions assuredly much more suggestive of *O. graminis* than of *O. herpotrichus.* Later, to be sure, Krüger (41), on the basis of actual identifications, concluded that *O. herpotrichus,* evidently in its true restricted sense, was, in spite of unimpressive pathogenicity under experimental conditions, the species generally concerned in Germany, and he held this conclusion to confirm an assumption to the same effect that he credited to Frank. Whatever assumptions may be credited to Frank would, however, judging from his published works, need to be predicated on an *O. herpotrichus* including within its limits both the *O. herpotrichus* and the *O. graminis* of other authors. In contrast to Krüger, McAlpine evidently fully understood Frank's ambiguous application of *O. herpotrichus.* This author, who at first (45) considered the "wheat-stem-killer (*Ophiobolus herpotrichus*)" in relation to "take-all," which had long been destructive to wheat in Australia, later (46) referred the disease to *O. graminis,* explaining that the fungus in question was identical with the wheat-stem-killer determined by Frank in 1894, as well as with the parasite found by Prillieux and Delacroix to be the cause of footrot in France. It is not apparent that the ambiguity was recognized by van Hall (30), whose extended account of a disease of wheat in Holland, which he attributed to the *tarwehalmdooder,* added to the reputation of *O. herpotrichus* as a serious pathogen.

This reputation, however, during the 3 succeeding decades, has not been fully sustained, though Crüger (5), as late as 1929, attributed to *Ophiobolus herpotrichus* an important rôle in the causation of foot disease. Owing to the unimpressive performance of the fungus under ordinary experimental conditions, Foex and Rosella (20, 21) in France, van de Laar (43) in The Netherlands and Schaffnit (69) in Germany, have come to regard it as a parasite of secondary importance. Rather curiously *O. herpotrichus* has, hitherto, apparently never been recorded as occurring in the United States, though the writer has found a fungus morphologically agreeing with and evidently referable to that species regularly occurring in great abundance on quack grass (*Agropyron repens* (L.) Beauv.) during April, May,

and early June in Maryland, the District of Columbia, and Virginia every year from 1922 to 1933, as well as near Madison, Wis., in the corresponding seasonal periods in 1919 and 1920, and on Long Island in and about Brooklyn, N. Y., in 1921. Evidently quack grass serves, at least in the northern part of this country, as a most congenial host of *O. herpotrichus,* and but for the low esteem in which this grass is held the reputed stem-killer of central and western Europe might long have been a familiar object to American pathologists. If, as seems not unlikely, the fungus is similarly frequent in northern and central Europe, the earlier reluctance of investigators in those regions to accept *O. graminis* as a separate species has some explanation. For Saccardo's citation of Agropyron as one of the two genera representative of the hosts of *O. graminis* could hardly have failed to convey somewhat the impression that the Ophiobolus occurring so abundantly and so frequently to the exclusion of any congeneric fungus on the very widely distributed member of that host genus must somehow have been the one on which his diagnosis was based.

Although the paper of Mangin (50) published in 1899, cast doubt on the causal relationship of *Ophiobolus graminis* to *piétin* in France, Delacroix (10), 2 years later, reestablished the position of the fungus as at least one of the parasites involved in that disease. In 1901 appeared also a report by Hori (36) attributing foot rot in Japan to *O. graminis,* and in 1904 McAlpine's account, already referred to, connecting the fungus with take-all in Australia. Thereafter the destructively pathogenic character of *O. graminis* appears never to have been seriously questioned. In the United States the fungus and the disease caused by it have been treated more especially in publication by Kirby (39, 40), Davis (9), and McKinney (47); while recent European testimony to the harmful effectiveness of *O. graminis* is given by Foex and Rosella, by van de Laar, by Schaffnit, and by Moritz (56).

Such degree of confusion as earlier obtained with respect to *Ophiobolus graminis* and *O. herpotrichus* had, it may be admitted, some excuse in the resemblance in habit between the 2 fungi. Similarities such as those expressed in the rather characteristic localization of the perithecia on the basal portions of grass culms, and in the presence of the superficial network of dark hyphae over the adjacent host substratum, constitute features of parallelism that would seem to indicate a narrowly congeneric relationship. Since these features are set forth also in Tullis' (74) account of *O. oryzinus* Sacc., the cause of a rice disease in Arkansas, and are sustained here besides by general similarities in dimensions of asci, as well as in dimensions, septation, and arrangement of ascospores, there is reason to believe that this parasite, too, is included in the same intimate relationship. *O. cariceti* (B. and Br.) Sacc., described originally from *Aira caespitosa* in

England, was found by Fitzpatrick, Thomas, and Kirby (18) to resemble the take-all fungus so closely in morphology of perithecium, asci, and ascospores that they held it to be the same organism and, accordingly, favored relegation of the name *O. graminis* to synonymy. However, as the only material of *O. cariceti* used in their comparisons consisted of microscope preparations and herbarium specimens no longer in living condition and, moreover, not entirely above question in regard to authenticity, the resemblances observed by them have, in conformity with representations made by McKinney, been generally deemed insufficient to justify so important a nomenclatorial change as the one they proposed. Of course, the possibility of such identity, earlier predicated by Berlese (2, v. 2, p. 119–120) in his citation of both *O. cariceti* and *O. graminis* as synonyms of *O. eucryptus* (B. and Br.) Sacc., is not yet to be dismissed; and, in any case, the resemblances referred to together with the similarity in position on the host, also pointed out by Fitzpatrick, Thomas, and Kirby, would seem to indicate a close relationship in the same series.

It appears probable that *Ophiobolus oryzae* described by Miyake (54) from rice in Japan may likewise belong in the same group with *O. graminis* and *O. herpotrichus,* even though its occurrence on the leaves and glumes of the host plant rather than on the basal parts fails to sustain the parallelism in parasitic habit. As to various other species of Ophiobolus recorded from dead graminaceous materials, as, for example, *O. medusae* E. and E. on culms of *Spartina* sp. in New Jersey, *O. festucae* Tracy and Earle on dead leaves of *Festuca* sp. in Colorado, *O. trichosporus* Ell. and Ev. on grass stems in Canada, *O.* (later *Ophiochaeta*) *trichellus* Bomm. Rouss. Sacc. on the lower leaves of *Psamma arenaria* Roem. and Schult. in Belgium, *O. seriatus* Syd. on dead stems of *Bambusa* sp. in the Philippines, *O. graffianus* Sacc. on the decaying caryopsis of *Coix lacrima-Jobi* L. in the Philippines, and *O. panici* Syd. on dead sheaths of *Panicum miliaceum* in the Philippines, the available information is too scanty for any conjecture as to their more intimate affinities. In the main, no doubt, these species owe their assignment to Ophiobolus much more to gross conformity with the broad Saccardian application of the genus, than to their degree of similarity to the 2 parasites through which the genus has become familiar to plant pathologists.

## HISTORICAL AND TAXONOMIC ASPECTS OF THE GENUS OPHIOBOLUS IN GENERAL

For, although *Ophiobolus graminis* and *O. herpotrichus* have occupied prominent places in mycological literature, their influence on the taxonomic history of the genus in which they are now included has remained unimportant. Indeed, in their present systematic setting, there is little historical

justification for regarding either the one or the other as a pivotal species. Ophiobolus was erected on a fungus found presumably in saprophytic relationship on *Carduus arvensis* Robs., and described as new by Riess under the name *Ophiobolus disseminans.* The newness of the species, to be sure, failed to convince contemporaneous writers some of whom, like Duby (63, no. 57), evidently considered it to be identical with the fungus that Sowerby (71, v. 3, pl. 394, fig. 3) had much earlier found "scattered on the stalk of a thistle" and described rather inadequately as *Sphaeria acuminata,* while others held it to be the same as Wallroth's (77) *Sphaeria carduorum,* of which the host substratum was only slightly more definitely indicated in the words *"ad caules Carduorum siccos."* Apart from the question of specific identity the fungus, according to Riess' description, was certainly not devoid of morphological distinctiveness, the many-septate ascospores being set forth as originating joined end to end in pairs, later to become separated individually at a commissure located between two swollen segments, and thus finally appear with the characteristic snakelike terminal swelling signalized in the generic name. Yet neither the rather astonishing development ascribed to the ascospores, which now would more likely be interpreted as representing disarticulation of a single ascospore into halves, nor the arresting nodosity of the inflated spore segments appears to have impressed writers of the time as constituting a character of generic significance. The disesteem in which both the genus and species launched by Riess soon came to be held is reflected in the use of the various binomials applied presumably to the same fungus, Rabenhorst (63, no. 530) citing it as *Rhaphidospora disseminans,* the Tulasne brothers as *Rhaphidophora carduorum* and Fuckel as *Rhaphidospora carduorum.*

In the same publication with the latter citation Fuckel further cited under Rhaphidospora, *R. rudis* (Riess) and *R. rubella* (P.), thereby in effect merging with Rhaphidospora, in addition to Ophiobolus, the 2 genera typified in these species. Of these 2 genera Entodesmium had been erected by Riess in 1854 on the newly described *E. rude,* the spores of which were set forth as being produced within clavate asci in chains of approximately 16 individuals,—a characterization now more aptly interpreted as referring to ascospores with approximately 15 septa disarticulating into their component segments. Leptospora, the second of the amalgamated genera, had been erected by Rabenhorst (62) in 1854 on a fungus he regarded as identical with the *Sphaeria porphyrogena* described as new by Tode (72) in 1791, but treated by Persoon (60) in 1801 as a variety of *Sphaeria rubella.* The distinctive feature setting his genus apart from Rhaphidospora was found, according to Rabenhorst, in the non-septate character of the ascospores, but as early as in 1863, Cesati and De Notaris (3), whether from doubt as to the reality or as to the significance of this imputed feature, cited the presumptive type species as *Rhaphidophora porphyrogena.*

In any case it is apparent that long before the publication of the *Sylloge fungorum* began, species of types sufficiently distinctive to have led earlier to the proposal of several separate genera had wisely or unwisely been brought into the same fold. Favored no doubt by the distinguished example set by the Tulasne brothers, the term Rhaphidophora apparently proposed by Cesati and De Notaris as a mere modification to avoid homonymy with Nees' genus, continued for about 2 decades to hold the field, though the earlier term reappeared now and then, as in Fuckel's meritorious work already cited. When it was finally realized that, curiously enough, the substituted term was a homonym no less than the one it displaced, Saccardo, as has been noted previously, revived Ophiobolus, applying it, of course, not only to the forms corresponding closely to the type on which it was erected, but also to the multitude of forms corresponding to the several other types with which its original type had in the meantime become agglutinated. In the second volume of the "Sylloge fungorum," although a number of species of the abandoned Rhaphidophora were committed to a new Saccardian genus Ophioceras, 63 species were compiled in Ophiobolus, and additions in subsequent volumes have more than trebled this number, even if allowance is made for the 7 species with more or less chaetose perithecia transferred to another Saccardian genus, Ophiochaeta, in 1895.

The very considerable increase in number of species assigned to it could hardly have failed to bring into relief the rather indiscriminating inclusiveness of the rehabilitated genus. Yet it was more from motives of taxonomic correctness than of expediency that Höhnel (34), in 1918, submitted a remedy in proposing to reestablish Entodesmium and Leptospora as genera independent of Ophiobolus, assignment of the various species to be governed by their similarity to the 3 historical types, *E. rude*, *L. poryphyrogona* and *O. acuminatus*, respectively. From his discussion it may be inferred that he regarded the complete disarticulation of the ascospores prevalent in the first of these types as an essential feature of Entodesmium, and median disarticulation between 2 swollen segments set forth in Riess' account of the second type, and also illustrated by Berlese in all the species figured by him under his Leptosphaeriopsis, as an essential feature of Ophiobolus. Each of these modes of disarticulation would seem to occur in only a relatively small proportion of the species compiled in Ophiobolus in the *Sylloge fungorum*, and it seems quite possible that their occurrence here may indeed constitute a feature representative of such thoroughgoing parallelism as distinguishes the members of natural genera. Leptospora, however, was apparently left by Höhnel in a more ambiguous state, for although it was to be constructed about *L. porphyrogona*, it was evidently to serve at the same time as repository for all forms included by Saccardo in Ophiobolus

that have spores, whether nodose or not nodose, that on maturation remain filamentous, that is, do not in any normal way become disarticulated. Manifestly this disposition would have transferred to Leptospora, with only relatively small reduction, the main mass of forms compiled in Ophiobolus. What Rabenhorst had emphasized as a distinctive feature of Leptospora, an imputed unicellular condition of the ascospores, was omitted from discussion, and not without justification from factual considerations, since Winter had described the spores of *O. porphyrogonus* in at least their later stages as being provided with many cross walls.

In a second paper Höhnel (35) applied to the genera under discussion the moot structural distinction between discrete fruiting bodies, of which he was a most persistent exponent. Finding the type species of Leptospora to be of "sphaeriaceous" structure, he made such structure a requirement for the retention of any fungus in the genus, the forms to be excluded by reason of their "dothideaceous" character being provided for in a new genus, Leptosporopsis. Since *Ophiobolus herpotrichus* also was revealed as being *"ganz deutlich dothideal,"* it may be inferred that inclusion in Leptosporopsis is to be counted among the events in the taxonomic history of that economically important species. Examination of *Entodesmium rude* likewise showed dothideaceous structure, whereas *O. acuminatus* was recognized as truly sphaeriaceous,—these findings, to be sure, not altering the narrow limits adopted by Höhnel for the 2 genera concerned in them. A rather considerable widening of the limits of Ophiobolus would seem to have been involved, however, in the later recommendation by Weese (78) that the species with 1 or 2 nodes and not given to disarticulation, which Höhnel had preferred to include in Leptospora, be transferred to the former genus.

The dispositions of Höhnel and of Weese appear to have proceeded from an assumption that if the species in Ophiobolus as applied by Saccardo were distributed judiciously mainly among the 3 historical genera and the new Leptosporopsis the resulting arrangement would somehow approximate, if not actually constitute, a natural one. Such as assumption would seem at least premature, for, on the basis of our present knowledge, only a relatively small number of the species can with tolerable certainty be assimilated as natural congeners to the type species of the historical genera, leaving, therefore, a large residue of unassimilated forms, which will continue to require an obviously collective genus. Now, since Ophiobolus has served as collective genus for a half century, its rehabilitation as a restricted genus would entail shifting of this function elsewhere with numerous changes in established binomials. Considerations of expediency may, therefore, be expected to militate strongly against such rehabilitation, possibly with the result that the group involved may again be integrated, even if

somewhat in defiance of strict priority, in Berlese's genus Leptosphaeriopsis. Revival of Leptospora and Entodesmium, neither of which has in any serious degree been compromised nomenclatorially, would entail no similar difficulty, and might well serve a good purpose, though, evidently, only if through study more detailed than routine examination of mostly dead herbarium specimens, their memberships were maintained in acceptable homogeneity.

Even if the elevation of Ophiochaeta to generic rank in 1895 relieved Ophiobolus as first applied by Saccardo of only relatively few species, it yet came to affect the nomenclature of both the take-all fungus and the reputed wheat stem killer. Hara (31), in 1916, made reference to the former of these parasites under the binomial *Ophiochaeta graminis* (Sacc.) Hara, and the latter was cited in 1930 by Clements and Shear (4, p. 277) under the binomial *O. herpotricha* (Fr.) Sacc. as the type species of the genus Ophiochaeta. The aptness of these dispositions would seem somewhat doubtful, for, judging from Berlese's figures of *Ophiochaeta helminthospora* (Rehm) Sacc. and of *Ophiochaeta penicillus* (Schmidt) Sacc. as well as from the illustrations of *Ophiochaeta chaetophora* (Crouan) Sacc. given by Malbranche and Niel (49), the perithecial setae of at least 3 of the forms Saccardo definitely referred to Ophiochaeta were represented by apparently rigid, rather stiffly radiating bristles. Bristles of such character are not readily identified with the flexible filaments that, although often attached here and there to the fruiting bodies of the two graminicolous parasites, have much the appearance of the hyphae that make up the mycelial plate. Berlese (2, v. 2, p. 127) recognized the distinction clearly and held that *O. herpotrichus* could not be referred to Ophiochaeta. Nor is it clear that Saccardo ever actually transferred *Ophiobolus herpotrichus* to Ophiochaeta, though his statement that this species must be more fully investigated indicates that he had considered such a transfer. In respect to shape of ascospores none of the 3 species of Ophiochaeta figured by Berlese and by Malbranche and Niel show close similarity to *Ophiobolus graminis* and *O. herpotrichus*, or for that matter, to one another. The genus as constituted gives the impression of being a heterogeneous assemblage of species and, therefore, as Weese intimated, might perhaps just as well have remained in Ophiobolus.

### MORPHOLOGICAL FEATURES OF THE HELICOID ASCIGEROUS SERIES

In 1925, through the discovery of the ascigerous stage of a fungus causing leaf spot of maize in the warmer regions of the world (13), the genus Ophiobolus in the broad Saccardian sense was brought into relation with a second field of phytopathological interest, certainly no less important than the first. The maize parasite directly concerned was one of the rather

numerous series of graminicolous species of Helminthosporium distinguished by conidia of typically elongated-elliptical outline that germinate by the production of 2 polar germ tubes, one from the apex, and the other from a narrow zone immediately surrounding the basal scar. As the thoroughgoing parallelism between most known members of the series was fairly obvious from their morphology as well as from their cultural and pathogenic behavior, it was inferred that, when perfect stages should be discovered for other members of the series, they very probably would show intimate morphological similarity with the one then described as *Ophiobolus heterostrophus* Drechsl. The correctness of this inference has since been confirmed. In 1927 Ito and Kuribayashi (38) described under the binomial *Ophiobolus miyabeanus* the ascigerous stage of *H. oryzae* B. de H. A year later Nisikado (57) described under the binomial *Ophiobolus kusanoi* the perfect stage of a fungus causing leaf blight of *Eragrostis major* Host, the conidial condition of which was referred to as *H. kusanoi.* In 1929 Ito and Kuribayashi (42) issued the description of *Ophiobolus sativus,* the ascigerous condition of the widely destructive *H. sativum* Pamm., King and Bakke and, in 1930, followed this with a description (37) of *Ophiobolus setariae,* the ascigerous stage of *H. setariae* Sawada. Martin (51), in a report published in 1931, stated that C. W. Carpenter had obtained the ascigerous stage of the fungus causing brown stripe of sugar cane, *H. stenospilum* Drechsl., and found it to be referable to Ophiobolus. As far as can be determined from the rather immature perithecial fructifications that the writer has obtained in cultures of *H. micropus* Drechsl., a parasite on *Paspalum boscianum* Flügge, widely distributed in the Southeastern States, this member of the series provides no departure from the morphological trend of the others.

This morphological trend is manifested conspicuously in pronounced helicoid arrangement of the ascospores within the ascus, taken together with rather unusual dimensional relationships that in part ensue therefrom. Even in *Ophiobolus kusanoi,* the smallest of the 5 ascigerous forms known in detail, the diameter of the ascospore (5 μ) exceeds the homologous dimension in the general run of species assigned to Ophiobolus, while the ranges of values given for *O. heterostrophus* (6–9 μ) are comparable with the asci rather than with the ascospores of a large proportion of the species compiled in the genus, and, indeed, in not a few cases even exceed them. Obviously, an ascus to contain a full complement of 8 such spores would, under any circumstances, need to be of proportionately greater diameter, but here, owing to the additional spatial requirements entailed in the helicoid arrangement of the spores, it needs to be of a diameter exceeding the corresponding dimension of Ophiobolus species generally, in a measure, more than proportional to the greater widths of the ascospores. It is not

surprising, therefore, that the published values for diameter of ascus in *O. heterostrophus* (24–28 μ), *O. setariae* (22–32 μ), *O. miyabeanus* (25–32 μ), and *O. sativus* (32–45 μ) are unequalled among the corresponding values given under the genus in the *Sylloge Fungorum,* and that only a few of the latter are comparable even with the values (14–18 μ) given for *O. kusanoi.* As the spiral arrangement permits the ascospores to attain a length considerably greater than that of the ascus, spores of unusual length (the maximum value given for this dimension in *O. miyabeanus,* 468 μ, would be regarded as extraordinary in any group of fungi), as well as of respectable diameter are borne in asci that, among scolecosporous groups, appear more remarkable for width than for length.

It is, of course, not to be asserted that the features largely giving character to the ascigerous series under consideration are entirely unknown among the numerous unsifted saprophytic forms compiled in Ophiobolus. As was pointed out earlier in the discussion of the parasite causing leaf spot of maize, helicoid arrangement of ascospores, besides being well illustrated in Berlese's figures of *Ophiobolus camptosporus* Sacc., has been attributed to both *Ophiobolus helicosporus* (B. and Br.) Sacc. and to *Ophiobolus galii* Rich., not to mention *Ophiobolus chaetophorus* (Crouan) Sacc., later transferred to Ophiochaeta. A fairly pronounced spiral condition with the direction of rotation reversing several times has been observed in especially well-developed material of a fungus collected near Clarendon, Virginia, with exceptionally long asci and longer ascospores, which, otherwise, in a general way, conforms morphologically to the species or series of species customarily referred to as *Ophiobolus porphyrogonus.* However, in none of these forms is the one feature of similarity sustained by others in a degree making for a parallelism indicative of close relationship with the helicoid graminicolous series. The single, median, nodose cells present in the spiral ascospores of *O. camptosporus* precludes a more complete similarity, and the dimensions of this species are hardly of an order to provide a close parallelism. As measurements of *O. chaetophorus, O. helicosporus* and *O. galii* have apparently never been published, information in regard to dimensional relationships is not here available. The very slender and conspicuously filamentous spores of *O. porphyrogonus* and of species closely related to it, differ so markedly from those of any of the helicoid graminicolous series that an intimate connection would seem rather definitely out of question. In many species of Ophiobolus a spiral tendency is often or occasionally evident in the upper portions of the ascospores, even where these structures are for the most part straight and disposed parallel to one another. Such a tendency represented, for example, in 2 specimens deposited in Mycological Collections of the Bureau of Plant Industry under covers labelled:

Sydow, Mycotheca germanica. 2343. *Ophiobolus ulnosporus* (Cke.) Sacc. Auf Stengeln von *Ballota nigra*, Brandenburg: Tamsel. 24. 7. 1927.

Fungi Dakotenses, Brenckle. 237. *Ophiobolus claviger* Harkn. On *Artemisia biennis*. Kulm, N. Dak., Aug. 18, 1913.

rarely entails more than a half turn of rather wide pitch, so that the ascospores are only infrequently if ever appreciably longer than the asci in which they are contained.

### ASEXUAL REPRODUCTION IN THE HELICOID SERIES AND IN OPHIOBOLUS

Although the series of strongly helicoid graminicolous species thus exhibits fairly distinctive features in the ascigerous stage, it will undoubtedly continue to be known mainly through the conidial stage achieved in the widely familiar group of forms within the genus Helminthosporium characterized by the special type of germination to which reference has been made. In general, the conidia here are produced abundantly on the natural substrata, and with few exceptions, also on artificial media, so that membership in the series is ordinarily very easily determined, even though identification of the individual species may require detailed comparisons. Yet the literature on Ophiobolus, outside of the publications dealing with *O. heterostrophus, O. kusanoi, O. miyabeanus, O. sativus, O. setariae*, and the brown-stripe parasite, makes no mention of any conidial condition suggestive of Helminthosporium. Indeed, relatively few contributions on subsidiary stages of any kind are to be found, and of these not all are in as complete harmony as might be desired.

The Tulasne brothers in their account of the fungus or fungi that they treated as *Rhaphidophora herpotricha* included as a stage in the life history set forth therein a pycnidial form producing elongated 6- to 10-celled stylospores measuring 25 to 35 μ long by 3.5 to 5 μ wide. It is not apparent that *Hendersonia herpotricha* Sacc. with 8-septate spores, 36 μ long and 6 μ wide, later cited by Saccardo as the pycnidial stage of *Ophiobolus herpotrichus*, was intended to have reference to the same sphaeropsidaceous form; or for that matter, whether so intended or not, that it actually had such reference. Hiltner (32), in 1912, asserted his continued belief in the association of a pycnidial form designated by him as *Hendersonia herpotrichoides* Sacc. that had appeared in 1894, following incubation in a damp chamber, on affected wheat plants originating in Saxony that year, with the Ophiobolus perithecia that had later developed thereon. Frank (23) considered it likely that a species of Phoma that he called *P. tritici* was associated with his *Weizenhalmtöter*. The genetic connection mentioned by Frank has no more received confirmation than has that mentioned by Hiltner; and in both cases uncertainty as to the identity of the species of Ophio-

bolus concerned is present as a disturbing consideration. Similarly dubious circumstances surround the report by Voges (76) of a connection between *O. herpotrichus* and *Acremonium alternatum* Link. On the one hand, as van de Laar strongly hinted, the measurements of length and maximum width of swollen ascospores, 66–74 × 4 μ, given by Voges, certainly indicate *O. graminis* rather than *O. herpotrichus;* and on the other, the relationship of *A. alternatum* to either of these species of Ophiobolus has never been confirmed. The pure cultures of *O. herpotrichus* isolated by the writer from different lots of quack grass collected in Clarendon, Va., have so far failed to reveal sporulation of any kind, sexual or asexual.

Subsidiary reproductive stages have been attributed also to *Ophiobolus graminis.* McAlpine (46), in 1904, was strongly inclined to regard a pycnidial form newly described by him under the name *Hendersonia graminis,* but later transferred to the genus Wojnowicia by Saccardo and D. Saccardo (68, v. 18, p. 367–368), as connected with the take-all fungus. McKinney and Johnson (48), however, on comparing the two forms in pure culture, found no close similarity between them, and were, therefore, led to question the probability of a genetic connection. Guyot's (29) studies later definitely established *Wojnowicia graminis* as a separate fungus. Mangin's claim of a connection between *O. graminis* and a species of Coniosporium somewhat doubtfully identified as *C. rhizophilum* (Preuss) Sacc. has likewise remained without confirmation. On the other hand, the production frequently of minute falcate sporidia in the germination of the ascospores of *O. graminis,* first reported by Mangin, was later confirmed by Foex (19), by Kirby (40), and by Foex and Rosella (21).

The Tulasne brothers attributed to the fungus discussed by them under the name *Rhaphidophora carduorum* pycnidia filled with cylindrical curved conidia as well as spermogonia producing cylindrical curved spermatia. Fuckel listed *Phoma acutum* and *Phoma complanatum* under *Rhaphidospora pellita, Phoma acutum* under *R. urticae,* and *Phoma dictamni* under *R. dictamni.* Höhnel (33), in 1915, stated that the asexual stages thus listed by Fuckel certainly did not belong to the ascigerous species to which they had been referred and held as questionable the association of pycnidial and spermogonial fungi with *Ophiobolus herpotrichus* and *O. carduorum* set forth by the Tulasne brothers. He considered as certain a connection between *O. porphyrogonus* and a pycnidial form, *Pleurophoma porphyrogena* Höhnel, having pycnidia measuring 150 to 250 μ in diameter that gave rise to rod-shaped hyaline spores 5 to 6.5 μ in length and 0.8 μ in width. In the *Sylloge Fungorum, Phoma rudis* Sacc. is mentioned, evidently on the authority of Karsten, as possibly a spermogonial phase and *Septoria rudis* Sacc. as possibly a pycnidial stage of *O. rudis; Phoma hesperidis* Sacc. is cited as the spermogonial phase of *O. hesperidis* Sacc.;

*Chalara monticellica* Sacc. is indicated as a conidial stage of *O. monticellicus* Sacc.; and reference is made to accessory reproductive phases in the diagnoses of *O. glomus* (B. and C.) Sacc., *O. rhagadoli* Passer. and *O. claviger* Harkn.

In the hope that some information might be obtained that would supply a clue as to the merit of these different references to subsidiary stages, the writer collected during May, 1932, living material of about a dozen species of Ophiobolus on the dead remains of various coarsely herbaceous plants. Pure cultures of each species were obtained readily by making dilution plate cultures from ascospores crushed out of the perithecia, care being taken to avoid contamination from admixed fungi. All showed satisfactory vegetative development on maize-meal decoction agar. Submerged sclerotia were developed in cultures of several forms of the type usually referred to as *O. porphyrogonus*. Scattered sclerotia representing probably immature fruiting bodies were formed in cultures of the well-characterized species occurring on stems of *Ambrosia trifida* L., the ascospores of which exhibit at their distal end a contour strongly suggestive of the head of a snake. The fungus was identified with the *O. anguillides* (Cooke) Sacc. reported on the same host substratum from Indiana by Fink and Fusan (17) as it was found to agree very well with specimens deposited in Mycological Collections of the Bureau of Plant Industry under the following labels:

> Fungi dakotensis, Brenckle. 236. *Ophiobolus anguillides* (Cke.) Sacc. On *Ambrosia trifida*. Aug. 24, 1913.
>
> Ascomycetes of Indiana prepared by Bruce Fink and Sylvia Fusan. *Ophiobolus anguillides* (Cooke) Sacc. Near Crawfordsville. On *Ambrosia trifida*. Number 381. 9–1–1917.

Actual sporulation was observed in cultures of only 2 of the fungi isolated, these having been obtained also from stems of *A. trifida*, on which substratum their fructifications occur year after year in very considerable abundance. Examination of specimens in Mycological Collections showed that the 2 fungi have apparently never been distinguished from one another, each being dealt with separately or both together as *O. fulgidus* (C. and P.) Sacc.

*Sphaeria fulgida* was described from Albany, N. Y., in 1875, evidently on the authority of Clinton and Peck (59) as follows:

"Perithecia gregarious, sometimes disposed in lines, soon free, globose, black, smooth, shining, scarcely papillate, .01′–.012′ in diameter, at length collapsed; asci clavate or cylindrical; spores filiform, curved or flexuous, multinucleate, at length multiseptate, colorless, .003′ long."

The host substratum was rather indefinitely indicated at the time as being "dead stems of herbs" but not much later, material collected by Peck

at Albany in June, 1879, and distributed under the binomial *Sphaeria fulgida* as No. 583 of Ellis North American Fungi was stated on the label to represent *Artemisia trifida*. It is not known to the writer whether an error was later acknowledged in the identification of the host, but in any case the specimen is now generally filed in herbaria as being of *Ambrosia trifida*, and is evidently thus considered in the host index of Farlow and Seymour (16) as well as in the more recent host index of Seymour (70). There can be little doubt that this disposition is correct, as *Artemisia trifida* Nutt. is not recorded as occurring in the eastern United States and as, moreover, the material is entirely similar to material known to be of *Ambrosia trifida*. A correction in identification of the host genus, impaired somewhat through an apparent garbling of the specific term, would seem to be implied in the citation of *Ambrosia trifolia* as host plant on the label of specimens of *Sphaeria fulgida* collected by Peck in June, 1880, and distributed as No. 1742 of de Thümen's Mycotheca Universalis.

The specimens of *Sphaeria fulgida*, distributed as No. 583 of North American Fungi, contain both of the species of Ophiobolus that were found to produce asexual reproductive stages in culture. From the close agreement with respect to size and shape of perithecium (Fig. 1, A, B), to length of ascus (Fig. 1, C), and to length and nearly colorless condition of ascospores (Fig. 1, D, *a–i*) it is evident that the description by Clinton and Peck was based on the smaller of the 2 species. It may be added that the ascospores that are rather regularly and typically 7-septate, and measure 55 to 95 μ in length by 3 to 3.5 μ in diameter, when freshly crushed out of the perithecium (Fig. 1, D, *a,b,c,f*) show such swelling in a water mount that a diameter of 4 to 6 μ (Fig. 1, D, *d,e,g,h,i*) is usually soon attained, the swelling often taking place even when the spores are retained within the ascus. On maize-meal-agar plate cultures the fungus gives rise to a scattering of rather dark, globose pycnidia (Fig. 1, E) from 0.1 mm. to 0.3 mm. in diameter, the exteriors of which reveal pseudoparenchymatous structure. The colorless hyaline pycnospores (Fig. 1, F), which are irregularly ellipsoidal in shape and measure 4 to 7 μ in length by 2.5 to 4 μ in width, are liberated from an ostiolar opening situated on the upper side of the pycnidium, sometimes in the center of a crater-like depression.

Although evidently the fungus with 7-septate, nearly colorless ascospores must be regarded as the one to which the binomial *Ophiobolus fulgidus* is correctly to be applied, it is not the one most frequently encountered under this name in herbarium collections. In Mycological Collections of the Bureau of Plant Industry this form has been recognized in only one specimen besides the specimen already referred to, that additional specimen being the one distributed as No. 3824 of the Fungi Columbiana, consisting of old stems of *Ambrosia trifida* collected by J. Dearness at London, On-

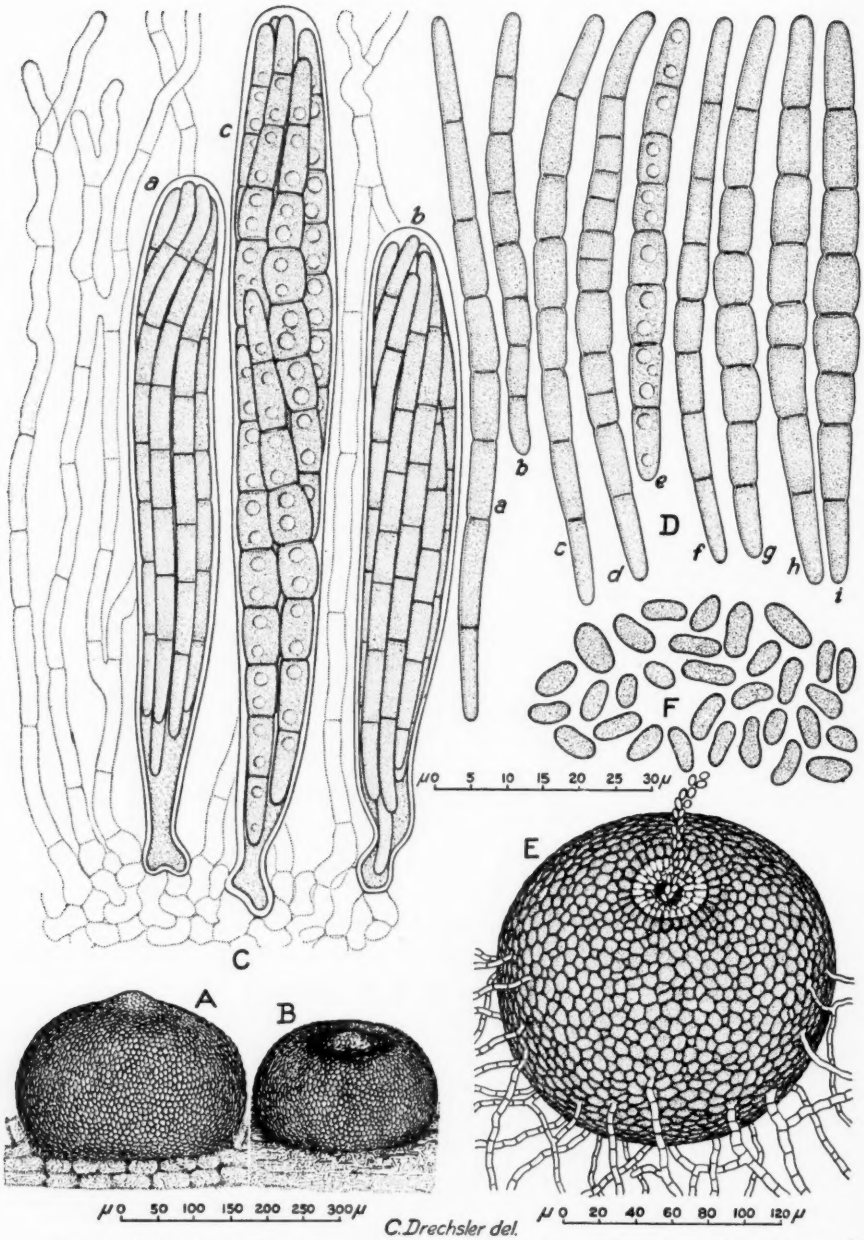


FIG. 1. *Ophiobolus fulgidus.* A. Perithecium on host substratum, viewed in profile. ×100. B. Perithecium on host substratum, showing apex somewhat collapsed. ×100. C. Asci, the spores within two, *a* and *b*, showing no swelling; the spores within *c* beginning to swell. ×1000. D. Ascospores, showing variation in size and shape, *a,b,c,* and *f* not swollen; *d,e,g,h,* and *i* somewhat swollen. ×1000. E. Pycnidium produced in pure culture. ×250. F. Pycnospores. ×1000.

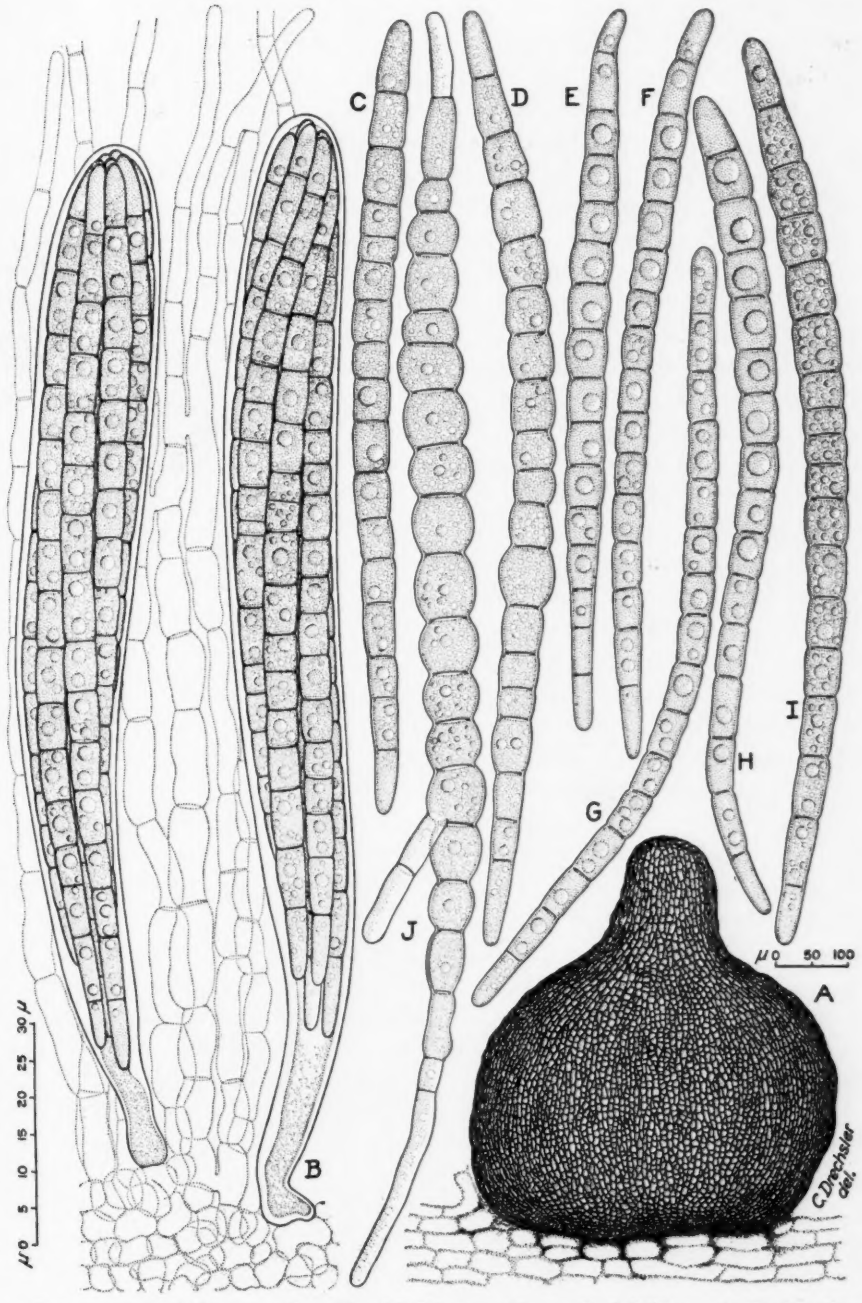


FIG. 2. *Ophiobolus* sp., often identified incorrectly as *O. fulgidus*. A. Perithecium on host substratum in profile. ×100. B. Asci, the ascospores not perceptibly swollen. ×1000. C–I. Free ascospores. ×1000. J. Germinating ascospore. ×1000.

tario, May, 1911. Microscope preparations of all other specimens designated as *O. fulgidus* and citing *A. trifida* as host, were found to represent the larger species included in Peck's specimen collected in June, 1879. The globose perithecia here measure mostly .35 to .5 mm. in diameter and are provided usually with a recognizable ostiolar beak, though this modification is generally not so prominent as in the fructification shown in figure 2, A. As there is little tendency toward collapse, the fruiting bodies of this species, which occur often in large numbers in close, somewhat linear arrangement, may be distinguished from those of the first form considered not only by their larger size but also by their more marked protrusion from the substratum. The asci (Fig. 2, B), on maturity, are 100 to 150 μ long by 13 to 17 μ wide and contain 8 spores of distinctly yellow coloration, which measure from 85 to 125 μ in length by 4 to 4.5 μ in diameter. The spores (Fig. 2, C–I) are usually and typically 15-septate, though the number of cross walls may be as low as 10 or 12 in unusually short individuals, or as high as 18 in unusually long ones. In a water mount living spores, after being liberated from the perithecium, swell to a diameter of 7 or 8 μ, evidently as a preliminary step toward germination, which ensues generally within a few hours (Fig. 2, J).

In pure culture on maize-meal-agar plates the fungus with 15-septate ascospores exhibits 2 subsidiary reproductive stages. Tawny pycnidia (Fig. 3, A, B) measuring from 25 to 125 μ in diameter are formed usually more or less superficially on the substratum, mostly in groups readily visible to the naked eye as flesh-colored masses. The pycnidia show externally a pseudoparenchymatous structure, though often and more especially in the smaller individuals (Fig. 3, B) the mycelial origin of this structure may be rather readily apparent. The orifice through which the spores are extruded in a cohesive mass is sometimes surrounded by a fringe of hyphal processes of variable lengths (Fig. 3, A). The pycnospores (Fig. 3, B), which are hyaline, colorless, and ellipsoidal, measure usually 2 to 3.5 μ in length by 1 μ in diameter and would seem, therefore, to be somewhat smaller than those of *Phyllosticta ambrosiae*, described by Davis (8) as causing a leaf spot of the giant ragweed in Wisconsin, though the difference in size is hardly sufficient to dismiss entirely the possibility of identity.

A second type of asexual reproduction (Fig. 3, C, D, E) is found in the production of spherical unicellular conidia in usually somewhat tortuous chains terminally on aerial hyphal elements that are mostly not well differentiated from the aerial mycelium generally. Rather curiously, the sporulating elements here are not of approximately uniform widths, as in most similar fungi, but vary in diameter from 1.5 to 4 μ. With this variation in the conidiophorous hyphae is associated a corresponding variation in the conidia, the more delicate hyphal elements bearing chains of conidia

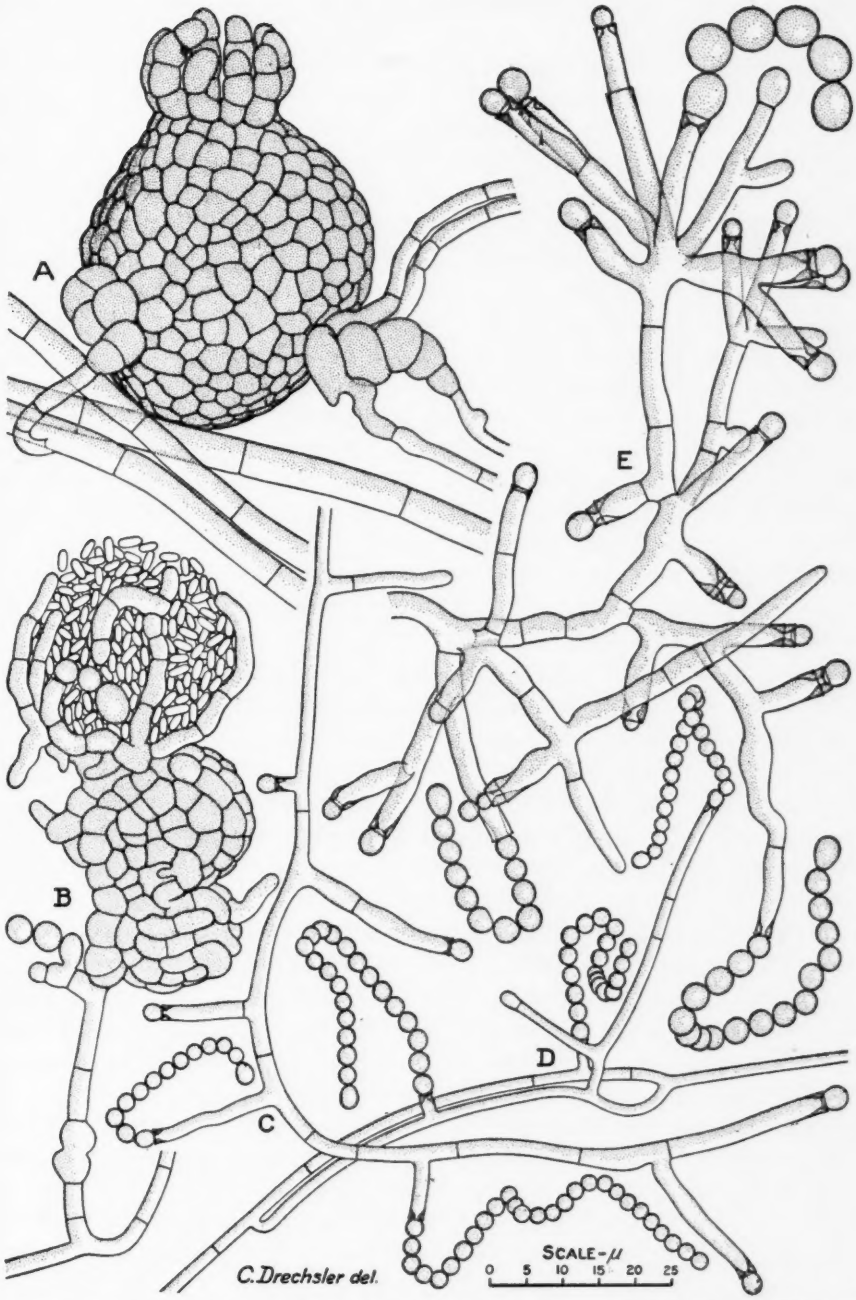


FIG. 3. *Ophiobolus* sp., often identified incorrectly as *O. fulgidus*. ×1000. A. Pycnidium of moderate size developed in pure culture. B. Small pycnidium. C–E. Aerial hyphae with sporiferous branches producing chains of aerial subspherical conidia, showing cuplike character of some sporulating tips, development of several successive rings at tips, and variation in diameters of conidia and conidiophorous branches; only the basal spore of a chain being shown in most cases.

2 μ in diameter, whereas the sturdier ones give rise to chains composed of individuals up to 6 μ in diameter. The production of each successive conidium following the first, entails a characteristic modification of the hyphal tip that becomes increasingly pronounced as the process is repeated again and again. On each occasion the peripheral wall at the tip for a short distance downward is slightly thickened on the inside, with the result that a thick rim soon comes to surround a narrowing isthmus through which the new spores are protruded as buds one after another. Often the isthmus comes to recede into the cup-like rim, so that the conidia are formed, as it were, partly endogenously, somewhat after the manner described by Andrus and Harter (1) for *Ceratostomella fimbriata* (Ell. and Hals.) Elliott, or by Woronin (80) for *Sordaria coprophila* De Not. Not infrequently, after the lumen of a sporulating tip has become much reduced, the hypha grows out vegetatively a short distance, establishing a new apex in which internal thickening again takes place. As a noticeable ring or collar remains to mark the position of the earlier tip, repetition of the process results in an annulated termination somewhat reminiscent of the conidiophores figured by Ducomet (15, pl. 31) for *Fusicladium dendriticum* (Wallr.) Fuckel, the conidial stage of *Venturia inequalis* (Cooke) Aderhold.

It would, in all probability, be going too far to interpret the tendency toward endogenous development of conidia just described as evidence of parallelism with the association supposed to obtain between *Chalara monticellica* and *Ophiobolus monticellicus*. As chains of conidia are usually much too fragile to withstand the handling incident to collection and removal to a laboratory, circumstances have been generally unfavorable for the recognition of catenulate sporulating stages as associated with ascigerous fructifications on material gathered in field or forest. On the other hand, as has been noted, homologues of the more durable pycnidia found produced in pure cultures of the 2 species cited under the binomial *Ophiobolus fulgidus*, have been recorded in the literature for various species of Ophiobolus as members of the form-genus Phoma. Genetic relationship with Phoma and allied sphaeropsidaceous types may thus represent an attribute shared rather widely among the many fungi occurring on the dead remains of coarsely herbaceous plants that provide the bulk of species subsumed under Ophiobolus. Since Phoma and the essentially similar Phyllosticta include numerous forms parasitic on the higher plants, it is possible that some of the unsifted species of Ophiobolus, now known as saprophytes, may ultimately be revealed as disease-producing organisms.

### THE HELICOID ASCIGEROUS SERIES SET APART AS A NEW GENUS COCHLIOBOLUS

In any case it appears significant that none of the dozen or more species of Ophiobolus grown in pure culture from ascospores gave rise to any co-

nidial stage resembling the series of graminicolous Helminthosporium forms with bipolar germination. As, conversely, this Helminthosporium series has never been found connected with species of Phoma or with any other sphaeropsidaceous stage, its separateness from what would seem to be the general run of species in Ophiobolus, appears fairly obvious, at least with respect to life-history associations. The series furthermore has apparently no intimate connection with the widely known plant pathogens, *O. graminis* and *O. herpotrichus,* even though its members, rather curiously, occur in part as parasites on the same graminaceous hosts, bringing about somewhat similar, though not identical, pathological changes. In order that confusion may be obviated between the helicoid ascigerous series corresponding to the Helminthosporium forms on the one hand and the take-all and stem-killing fungi on the other, it seems expedient to set apart the former as a separate genus for which a term referring to the spiral disposition of the ascospores is proposed.

**Cochliobolus,** gen. nov.

Perithecia sparsa, globulosa, coriaceo-membranacea, ostiolo papillato, atra, glabra aut hyphis sterilis vel hyphis conidiophoris vestita. Asci cylindracei, brevi-stipitati, saepius ad maturitatem plus minusve inflati, typice octospori. Sporidia filiformia, multiseptata, hyalina vel praeprimis ad maturitatem flavo-fumaginea, stipata, vehementer et regulariter spiraliter convoluta. Conidiophora rigidula, simplicia vel ramosa, saepius fusca, pluriseptata, geniculata. Conidia typice elongato-ellipsoidea, recta vel curvata, pluriseptata, hyalino-fumiginea vel fusca saepius olivacea, hyphis duabus germinantia quarum altera ex apice altera hilum juxta emergens.

Perithecia scattered, black, submembranaceous to subcoriaceous, smooth or covered more or less with flexuous vegetative filaments or with somewhat more bristling conidiophorous hyphae, globose, usually with evident paraboloid or short cylindrical ostiolar beak. Asci subcylindrical, short-stipitate, often becoming more or less distended especially previous to dehiscence. Ascospores 1 to 8 in number, colorless or especially at maturity somewhat colored, filamentous, provided with many septa, crowded, disposed in strongly helicoid arrangement. Conidiophore simple or somewhat branched, mostly olivaceous, septate, producing first conidium some distance from base and following repeated subterminal elongation successive conidia at intervals later often marked by geniculations. Conidia elongated-ellipsoidal or sometimes somewhat fusoid, straight or curved, nearly colorless to deep olivaceous, provided with plural septa, germinating by the production of 2 polar germ-tubes, one from the apex and the other from a zone immediately surrounding the basal scar.

The type species is **Cochliobolus heterostrophus,** nov. comb. (= *Ophio-*

*bolus heterostrophus* Drechsl., Jour. Agr. Research, v. 31, p. 701–726, 1925; and Phytopathology, v. 17, p. 414, 1927).

The new genus thus defined in both its sexual and asexual stages is intended to include only species that through a thoroughgoing parallelism in morphology and life history can safely be regarded as narrowly and naturally congeneric with the parasite causing leaf spot of maize designated as the type species. As undoubtedly the ascigerous stages of more Helminthosporium forms with bipolar germination will become known, in addition to the half dozen already described or reported in the literature, the scope of Cochliobolus with respect to number of likely members would seem, even with scrupulous application, to be a sufficiently ample one. Beside the 18 graminicolous species occurring in Japan that were cited by Nisikado (58) under his conidial subgenus eu-Helminthosporium, with which in the main the proposed ascigerous genus corresponds, the literature of other countries contains references to an approximately equal number of additional Helminthosporium forms parasitic on graminaceous hosts that are evidently referable to the same category. The writer has encountered on hosts other than grasses species that have every appearance of belonging here, the rather frequent occurrence of such forms, especially on dead or fading foliage of sedges, giving grounds for a suspicion that the destructiveness occasioned by the series of fungi in question among the Gramineae may in some measure be duplicated among the Cyperaceae. Conidial forms apparently of the same series have also been recognized on dead materials representing a goodly variety of woody and herbaceous plants, after incubation in a damp chamber, their occurrence, on the whole, indicating a fairly widespread distribution in nature, apparently in saprophytic as well as parasitic relationships.

On the other hand it is hardly to be assumed that the numerous species described from woody and herbaceous substrata that make up the main mass of forms compiled in Helminthosporium have representation in the series achieving the perfect stage in Cochliobolus in any proportion approximating the representation here of the graminicolous species of Helminthosporium. For, judging from the illustrations given by different authors, it appears rather improbable that either of the 2 main series that largely account for the graminicolous species account in comparable measure for the large body of unsifted fungi included under Helminthosporium in the *Sylloge Fungorum*. It may not be superfluous to emphasize that not all of even the graminicolous species can be assigned to one or the other of the 2 main series. In mode of germination and production of a Hormodendron-like sporulating stage, *Helminthosporium giganteum* H. and W., as was pointed out earlier (14), certainly departs widely from the series having its ascigerous phase in Pyrenophora Fries. The demonstration recently by

Tullis (75) of a connection between *H. sigmoideum* Cav. and *Leptosphaeria salvinii* Catt. has brought to light a third ascomycetous relationship within the group. The short sterigmata on the conidiophores of *H. sigmoideum* figured by Tullis, on which the conidia are borne directly, have no counterpart among the generality of graminicolous species of Helminthosporium and may, therefore, have value as a characteristic indicating similar pleomorphic association in other conidial forms. Nor are spherical sclerotia of the type represented in *Sclerotium oryzae* Catt., which is now revealed by Tullis as another phase in the life cycle of the same species of Leptosphaeria, known among the members of the 2 main graminicolous series. Even the decidedly different cylindrical sclerotia produced in culture by *H. cyclops* Drechsl. arouse misgivings concerning the affinities of that species, though the discrete conidiophores and the conidia here conform tolerably well in morphology to the homologous structures of known species of Cochliobolus. Apparently similar columnar or filamentous sclerotia occur frequently in a series of species, including forms like *H. geniculatum* T. and E. and *H. inequale* Shear, that are distinguished further by smaller, often geniculate conidia, the swollen middle cells of which are usually darker than the end segments. Whether the series, which is often recognized on grounds well set forth by Mason (52), as a separate genus, Acrothecium Preuss (?), falls outside the scope of Cochliobolus, must remain in doubt until the ascigerous condition of one of its members has been discovered. Of the arrangement of the conidia in a terminal whorl, which is customarily cited as the most distinctive feature of the series or genus, the present writer has seen little evidence, since, in all cultures of the group examined by him, the arrangement was found exactly as in cultures, for example, of *H. sativum*. Indeed, to the extent to which any difference was observed, it was rather the smaller species that produced the more prolonged and more abundantly laden spike-like racemose sporophores.

In characterizing the perithecium in Cochliobolus as glabrous or as bearing sterile hyphae or even conidiophores, it is intended to dispose of a feature that has been brought into undue relief through the Saccardian practice of using the presence of setae on the fruiting body, or their absence therefrom, for the separation of genera. The criterion very probably acquired its impetus by virtue of its actual merit in instances where the setae represent special structures pertaining definitely to the reproductive body in question; and it undoubtedly continues to serve well wherever such special structures are involved. However, in application even the occasional presence of adventitious hyphal filaments has sometimes been interpreted as constituting a setose condition. In *C. heterostrophus* the perithecium in some cases is thoroughly glabrous, but in other cases it shows a variable development of undifferentiated hyphae as well as of conidio-

phorous filaments. Such development should, of course, not be interpreted as pertaining to the sexual stage at all, but rather as intrusion of the vegetative and the asexual reproductive phases, brought on by appropriate environmental conditions.

### HELMINTHOSPORIUM, PYRENOPHORA, AND PLEOSPORA

In this connection it may be appropriate to consider the distinction between the genera Pyrenophora and Pleospora Rab. In an earlier paper (12) the writer transferred the ascigerous stages of *Helminthosporium teres* Sacc., *H. tritici-repentis* Died. and *H. bromi* Died. from the latter to the former genus, though the transfer entailed the making of several new combinations. The change was made not without the knowledge that as competent an authority as Winter (79) had regarded as inadequate for the separate maintenance of the 2 genera, the distinction based on the presence or absence of setae, emphasized in the works of Saccardo (68) and of Lindau (44). Indeed, it was recognized that Winter's view regarding the inadequacy of this distinction in itself had very considerable justification, for, although sterile bristles not readily to be confused with vegetative hyphae were usually found on the perithecia of the 3 fungi mentioned, their irregular occurrence and occasional suppression argued against their interpretation as structures of primary significance. A circumstance disturbing to Winter's dispositions was apparent in that, if the 2 genera were to be treated as a single genus, the term Pyrenophora dating from 1849 might deserve preference on the score of priority over the term Pleospora, originating in 1857.

However, when the 2 genera are considered in regard to the life history and the perithecial structure represented in each of their respective types, more substantial reasons for their separation are revealed. The genus Pleospora was erected on *P. herbarum* (Pers.) Rab., a species that has become widely known in both saprophytic and parasitic relationships. Since the time when Miyabe (53) definitely determined the parasite on onions (*Allium cepa* L.), which host has apparently as good a claim as any to be considered the type source of the species, to be connected with *Macrosporium parasiticum* Thüm., the same or at least very similar conidial form has been repeatedly shown to be the asexual reproductive stage of the fungus by other workers. In short, an altogether sufficient body of evidence—which the present writer had occasion to corroborate successfully—has accumulated, showing that *P. herbarum* has as its conidial stage a Macrosporium species of the "sarcinula" type, for which Mason submitted the name *M. sarcinula* Berk. emend Bolle as the most acceptable one.

When the genus Pyrenophora was established, Fries (27) listed in it 3 species under the following names: *P. paradoxa* Fr., *P. inclusa* (Kunz sub

Sclerot.) and *P. phaeocomes* Reb. Concerning the first and second of these species not much information has ever become available, and it is very doubtful whether either of the 2 names could now be definitely referred to any particular fungus. As a result *P. phaeocomes* is left as the obvious type of the genus and is properly listed as type by Clements and Shear. The sclerotia of this fungus were described by Rebentisch (64) in 1804 as *Sphaeria phaeocomes,* the host substratum mentioned being withering leaves of *Holcus mollis* L. The habit sketch supplementing the description, showing imbedded sclerotia distributed over the blade of the grass leaf, like the more recent habit sketch of Berlese, is strongly reminiscent of withered leaves of *Bromus inermis* Leyss. bearing sclerotia of *P. bromi,* such as may be observed in southern Wisconsin during late summer and autumn. Herbarium specimens in the Mycological Collections of the Bureau of Plant Industry, representing collections made in recent times in various European localities and consisting of leaves of *Holcus lanatus* L. and *Holcus mollis* with sclerotia in them scattered much after the manner illustrated in Rebentisch's figures, leave no doubt that the relatively ancient species enjoys an exceptionally consistent application. Though the fungus might well be expected to occur on *H. lanatus* in some of our northern States, the writer has not succeeded in obtaining freshly collected living material of it and, therefore, has been unable to ascertain more precisely the details of its life history. Fuckel, who presumably saw favorable material, stated that its conidial stage was similar to that of *Pyrenophora relicina,* without, however, giving further particulars concerning the morphology of the conidial stage of either species. Yet, since he cited *Pleospora polytricha* Tul. as a synonym of his *Pyrenophora relicina,* his statement is manifestly to be interpreted as implying resemblance to the conidial stage set forth in the account of *Pleospora polytricha* given by the French mycologists. The illustrations of conidia and conidiophores in the plate supplementing this account are perhaps most readily referred to a Helminthosporium occurring abundantly every year on overwintered oat straw during May and June in Maryland, Delaware, and Virginia in association with a Pyrenophora having ascospores with 5 transverse septa. Though the substratum obviously suggests identity of this Helminthosporium with *H. avenae* Eidem, the conidia on the overwintered straw are in general smaller (40–100 $\mu \times 12$–15 $\mu$), darker (sometimes olivaceous), and less frequently (usually 2 to 7 times) septate than conidia from the leaf-stripe lesions developing on the foliage of oats, often in some abundance, late in June and early in July. In any case the illustrations of the Tulasne brothers leave no doubt that the conidial stage of their *Pleospora polytricha* belongs to the Helminthosporium series with indiscriminate germination from any or all segments, familiar as pathogens concerned in the causation of important

diseases of cereal-crop plants. Fuckel's statement thus testifies to a parallelism in the asexual reproductive stage between Rebentisch's historically important species and the disease-producing series under consideration.

The supplementary parallelism in the sexual stage is expressed especially in the hard sclerotioid texture of the immature perithecium, and in the usually delayed and somewhat protracted development of the asci within. These features were very properly cited by Fuckel in his definition of Pyrenophora, which must be reckoned as a very happy one, whether or not the texture and development in question, together with peculiarities in internal perithecial structure associated therewith, can be regarded as requiring inclusion of the genus in the Dothidiaceae, or, for that matter, in the Pseudosphaeriaceae of some more recent writers. The intimate relationship of the species which Fuckel definitely referred to the genus, including besides *P. phaeocomes* and *P. relicina*, the collective *P. trichostoma* (Fr.) Fuckel (from which latter Diedicke (11) later correctly segregated a number of integral species, among them the ascigerous stages of *Helminthosporium teres, H. bromi,* and *H. tritici-repentis*), was recognized by Saccardo and Winter in retaining them in a subgenus eu-Pyrenophora. When Saccardo amalgamated this distinctive group with the more miscellaneous nonsclerotioid setose forms in his subgenus Chaetoplea, the larger genus Pyrenophora that resulted manifestly represented a collective genus. The recent elevation of Chaetoplea to generic rank by Clements (4, p. 275) remedies this undesirable disposition, in effecting the rehabilitation of Pyrenophora as a natural genus in the sense in which it was defined and applied by Fuckel. And to this sense it would seem to be highly desirable that future usage and application should rigorously conform.

## SUMMARY

Of the 2 graminicolous species, *Ophiobolus herpotrichus* and *O. graminis*, through which the genus Ophiobolus became widely known among plant pathologists, the former long retained a reputation as a pathogen in excess of its actual destructiveness. The description of *O. graminis* by Saccardo apparently remained for many years unknown to the earlier observers of cereal-crop diseases, and later, when the description must have been known, Frank, presumably from doubts as to the separateness of the fungus in question from the older *O. herpotrichus*, apparently assigned Ophiobolus injury in general to the latter species, and brought the composite concept into prominence through the term *Weizenhalmtöter*. The formidable parasitic character implied in the term adhered to the binomial with which Frank brought it into association, long after the separateness of the 2 species which this author manifestly considered identical, was generally admitted.

*Ophiobolus herpotrichus,* though not hitherto reported in the United States, has been found to occur very generally and in quantity on dead stems of quack grass during April, May, and early June in Wisconsin, New York, Maryland, and Virginia.

*Ophiobolus herpotrichus* and *O. graminis* are probably to be regarded as narrowly related members of a series to which *O. oryzinus* also seems to belong. This series would seem to have no intimate relationship to the generality of forms compiled in Ophiobolus nor to the apparently equally independent and phytopathologically important helicoid ascigerous series corresponding to the series of forms in Helminthosporium with bipolar germination.

Of the general run of species in Ophiobolus, more asexual stages are recorded in Phoma than in any other genus. Corroborative evidence of the reality of this pleomorphic association was found in the production of pycnidia in pure cultures of 2 species from dead stems of the giant ragweed, both of which have been treated—the smaller one correctly, the larger one incorrectly, even if more frequently—under the binomial *O. fulgidus.* Of more than a dozen miscellaneous Ophiobolus species from various herbaceous host substrata, none gave rise in culture to a Helminthosporium stage and none showed any family resemblance in cultural and mycelial habit to species of Helminthosporium. It is concluded, therefore, that the helicoid ascigerous series constitutes a separate natural genus, which is accordingly described under the name Cochliobolus; the fungus causing leaf spot of maize, *C. heterostrophus,* being designated as type species.

The filamentous outgrowths sometimes present on perithecia of Cochliobolus are to be regarded as intrusions of the vegetative and the asexual reproductive stages, rather than as pertaining to the ascigerous stage itself. Excessive emphasis on the presence or absence of setose outgrowths as criterion for distinguishing Pyrenophora and Pleospora has obscured the much more important difference in life histories present here. Recently rehabilitated as a natural genus through the elevation of Chaetoplea to generic rank, Pyrenophora again conforms to Fuckel's definition, being properly reserved for the hard sclerotioid perithecial forms having their asexual stages in the Helminthosporium series with indiscriminate germination corresponding broadly with the subgenus Cylindro-Helminthosporium of Nisikado and the genus Drechslera of Ito.

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# INHERITANCE OF ABILITY TO LOCALIZE TOBACCO-MOSAIC VIRUS

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There have been many reports of inheritance of resistance to infection with virus diseases in plants. The following may be cited as examples. Allard (1, 2) noted that the hybrid *Nicotiana tabacum* L. × *glutinosa* L.[2] failed to show the mottling which is characteristic of *N. tabacum* when infected with tobacco mosaic, responding instead with necrosis like that of *N. glutinosa*. Smith (12) crossed mosaic-susceptible commercial varieties of spinach with a Manchurian spinach which escaped mosaic in the field, and found that resistance to infection was transmitted to the hybrid seedlings, and could be strengthened by selection among the hybrid strains. Lesley (8, 9) observed a tendency to escape curly-top infection in certain dwarf tomato varieties. He failed to transmit this tendency to standard, *i.e.*, non-dwarf, progenies after hybridization of resistant dwarfs with susceptible commercial varieties of standard habit, but recovered it in dwarf strains derived from hybrids. Bennett (3), in studying alpha-curl virus in the hybrid raspberry variety, Columbian, found recovery from disease symptoms, with disappearance of virus and renewal of susceptibility. It was believed that this hybrid was a seedling from the susceptible variety Cuthbert, pollinated by the immune variety Gregg. Mackie and Esau (10) reported resistance to curly top partially correlated with red color in bean hybrids. Porter (11) found hybrids between mosaic-resistant Chinese Long cucumbers and commercial susceptible strains to be susceptible, but less stunted than the susceptible parents.

The present paper describes a dominant Mendelian genetic factor that causes localization of tobacco-mosaic virus in inoculated leaves of garden pepper, *Capsicum frutescens* L., and a similar factor in each of certain Nicotiana L. and Solanum L. species. Plants possessing the hereditary factor for localization are susceptible to infection with tobacco-mosaic virus, but resistant to spread of virus in their tissues. It is believed that this is the first description of such a genetic factor affecting a virus disease in its plant hosts. The virus of streak disease of maize is influenced while in its insect host, the leafhopper *Cicadulina mbila* Naude, by a dominant, sex-linked, Mendelian factor, described by Storey (13). This dominant factor in the leafhopper confers ability to act as a vector of the disease. In a recent paper Storey (14) has thrown light on the nature of this hereditary charac-

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[2] *Nicotiana glutinosa* was referred to by Allard as *Nicotiana viscosum*.

ter by demonstrating that the recessive-type, *i.e.*, inactive, insects may be made infective by wounding the wall of the intestine immediately before or after the insects are allowed to feed on diseased plant material, or by introducing virus directly into the insects' blood.

### CAPSICUM FRUTESCENS

#### Types of Response of Pepper Varieties to Infection with Typical Tobacco-mosaic Virus

The majority of garden pepper varieties respond to infection with typical distorting-type tobacco-mosaic virus (5, p. 847) by mottling. In these varieties, yellowish primary lesions develop about 3 days after inoculation, and are followed by chlorosis of young leaves, with a prolonged period of reduced growth rate for the plant as a whole. Sometimes partial defoliation occurs. In time many mottled and distorted new leaves are formed. The yield of fruit of mottled plants is much less than that of healthy plants. As examples of varieties which give a mottling response, there may be cited the *Capsicum frutescens* varieties California Wonder, Celestial, Chinese Giant, Coral Gem Bouquet, Early Giant, Giant Crimson, Golden Dawn, Golden Queen, Hungarian, Large Bell, Oshkosh, Pimiento, Red Cherry, Red Japan Cluster, Ruby King, Spanish Monstrous, Upright Sweet Salad, and World Beater.

A few pepper varieties, on the other hand, respond by localized necrosis, without systemic mottling. Among the varieties tested, only two have consistently shown sharply-defined necrosis at the site of inoculation. These are *Capsicum frutescens* var. Tabasco, and *C. frutescens* var. *minimum* (previously grown under the name of *C. minimum* Blanco, but differing from common varieties of *C. frutescens* no more than these differ among themselves). These peppers are immune from the serious systemic effects of tobacco mosaic, although they are highly susceptible to infection. Many small necrotic lesions develop in inoculated leaves on the 2nd or 3rd day after inoculation. Virus may be recovered from these lesions, but not from green areas between lesions or from uninoculated parts of infected plants. Affected leaves drop off, usually between the 2nd and 5th days. Leaves with many lesions abscise earlier than those with few. The plants are free of virus as soon as all inoculated leaves have fallen. They are susceptible to repeated infection, but eliminate the virus promptly and grow essentially as normal plants.

Peppers giving these two kinds of response will be referred to as mottling-type and necrotic-type plants, respectively. For the purpose of studying the response of hybrids between mottling-type and necrotic-type plants, crosses were made between the mottling-type varieties Ruby King and Golden Dawn, and the two above-described necrotic-type varieties.

Response of Hybrids to Infection with Typical Tobacco-mosaic Virus

*Capsicum frutescens var. Tabasco × var. Ruby King.*—Pollen from a plant of the mottling-type variety, Ruby King, was applied to stigmas of emasculated flowers of the necrotic-type variety, Tabasco. Many seeds were secured, but few germinated. Only 2 seedlings survived to blossom; both of these plants showed necrotic primary lesions after inoculation of leaves with typical distorting-type tobacco-mosaic virus. One of the 2 plants set no seed; the other bore many fruits, which were larger than those of the seed parent Tabasco, and when ripe partly pendant, like those of the pollen parent, Ruby King.

From seeds of the one original fertile plant, 48 second-generation seedlings were grown. Of these, 37 showed only necrotic primary lesions after inoculation with tobacco-mosaic virus, but 11 failed to show necrotic primary lesions and subsequently mottled. This reappearance of the mottling response in approximately a fourth of the second-generation hybrid plants suggested the possibility that a simple Mendelian dominant factor might be responsible for ability to localize the virus. The number of observed plants, however, was so small that this approach to a simple Mendelian ratio might have been accidental.

After the symptoms had been observed, the 48 infected plants were all set outdoors in a row in a garden. No attempt was made to prevent cross-pollination by insects. Eleven plants remained mottled and of high tobacco-mosaic virus content until autumn; the remaining 37, having lost their inoculated leaves, proved free of tobacco-mosaic virus. Some of the plants bore many fruits, others few or more. The 11 mottling-type plants bore comparatively few fruits, and, as later results showed, had probably received some pollen from nearby necrotic-type plants. Enough seeds were collected to grow seedlings from 36 necrotic-type plants and from 8 mottling-type plants.

Eleven of the 36 sets of seedlings from necrotic-type plants contained only necrotic-type plants, 1370 in all; the remaining 25 sets of seedlings from necrotic-type plants, a total of 2611, contained both necrotic-type and mottling-type individuals in each set in ratios approximating 3:1 (3.23:1 in the group as a whole). This seemed to indicate that 25 of the 36 necrotic-type $F_2$ plants had been heterozygous and 11 homozygous for a virus-localizing character. Combining these with the 11 plants that possessed the apparent recessive character, the ratios 11:25:11 were obtained, with one necrotic-type $F_2$ plant unclassified because of lack of seed. This is in good agreement with the expected 1:2:1 ratios characteristic of inheritance of a unit character in a second hybrid generation.

The 8 sets of seedlings from mottling plants did not contain mottling-type individuals only, as had been expected, but included some necrotic-

type plants. The ratio was 50 necrotic-type to 208 mottling-type plants, or 0.24 : 1. This ratio is small, and it is believed that the necrotic-type plants occurring in these progenies were the result of natural cross-pollination from necrotic-type plants growing nearby. To eliminate such accidental cross-pollination, 36 mottling-type seedlings from a heterozygous necrotic-type seed-parent were grown in an isolated greenhouse. Seed was obtained from 20 of these, the others setting no fruit. From this seed, 347 seedlings were grown. The seedlings were inoculated with tobacco-mosaic virus; none of them showed primary necrosis, but all developed mottling-type infections.

These results indicate beyond reasonable doubt that in *Capsicum frutescens* the ability to localize the virus of tobacco mosaic is transmitted in inheritance as a simple Mendelian dominant character, the lack of which allows movement of the virus to distant parts of inoculated plants, with resultant blanching, mottling, stunting, and reduction of crop, the symptoms commonly associated with the presence of the mottling type of tobacco-mosaic infection in peppers. The response of heterozygotes was indistinguishable from that of necrotic-type homozygotes. Lesions on inoculated cotyledons of necrotic-type plants resembled those on true leaves. The inoculation of variegated leaves, which occasionally appear on necrotic-type plants, produced approximately as many characteristic necrotic lesions in white, apparently chlorophyll-free, areas as in normal green areas.

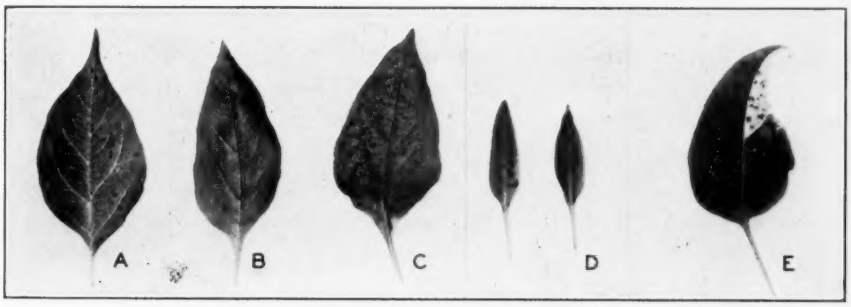


FIG. 1. Leaves of *Capsicum frutescens* inoculated with tobacco-mosaic virus. A. Necrotic primary lesions on leaf of a homozygous plant of constitution, *LL*. B. Lack of such lesions on a similarly treated leaf of a recessive individual, *ll*. C. Necrotic primary lesions on leaf of heterozygous individual, *Ll*. D. Similar necrotic primary lesions on cotyledons of necrotic-type plants. E. Similar lesions in both white and green areas of a variegated leaf.

Figure 1, (A), represents necrotic lesions on an inoculated leaf of a homozygous necrotic-type plant; (B), lack of such lesions on a similarly treated leaf of a mottling-type plant; (C), necrotic lesions on an inoculated

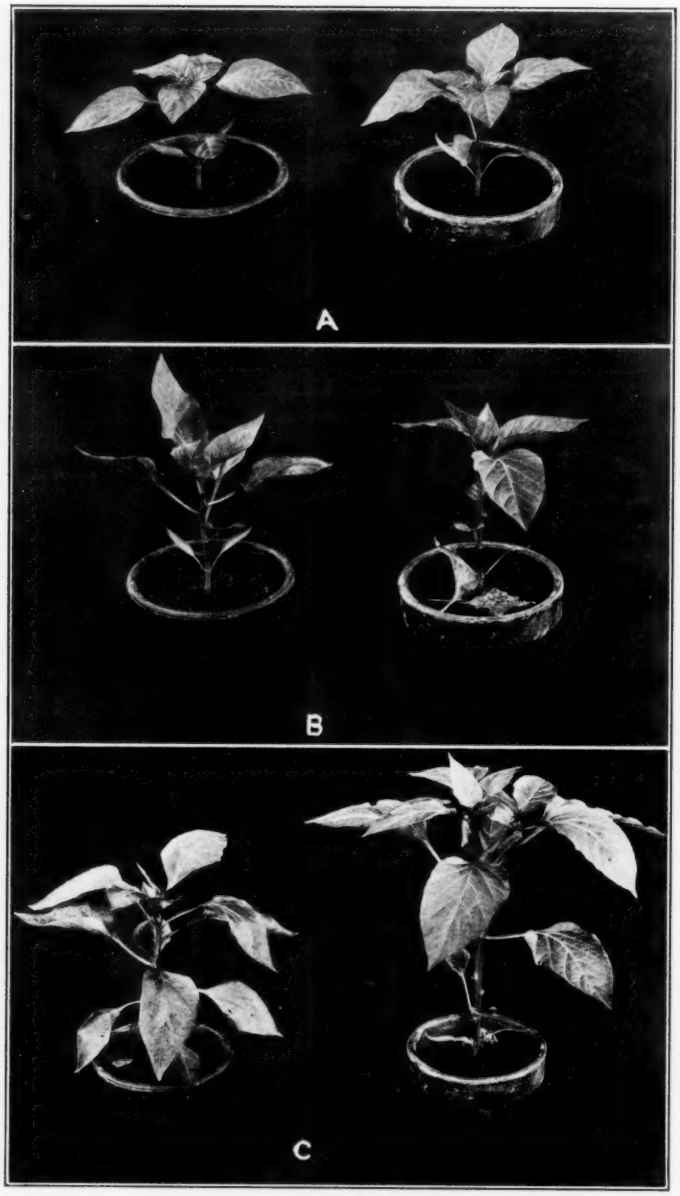


FIG. 2. Two plants of *Capsicum frutescens*, inoculated with tobacco-mosaic virus. The first was a mottling-type plant and the second, a necrotic-type. A. 3 days after inoculation of 2 leaves of each. B. 7 days after inoculation. Inoculated leaves had fallen from necrotic-type plant, freeing it from virus. C. 16 days after inoculation. Mottling-type plant was stunted and mottled. Necrotic-type one was large, without symptoms, and free of virus.

leaf of a heterozygous individual; (D), necrotic primary lesions on cotyledons; and (E), similar primary lesions in both white and green areas of a variegated leaf.

In figure 2 there are represented 2 plants, the first a mottling-type plant, the second a necrotic-type plant, (A) 3 days after inoculation of 2 leaves of each, (B) 7 days after inoculation, when the necrotic-type plant had lost its infected leaves, and (C) 16 days after inoculation, the mottling-type plant being then stunted and mottled, the necrotic-type plant larger, without symptoms of disease, and free of virus.

A fourth hybrid generation was grown, using in most cases seed from commercial, mottling-type *Capsicum frutescens* varieties, such as Ruby King, Golden Dawn, and California Wonder, fertilized with pollen from the third-generation, necrotic-type plants. The seed parents were thus homozygous for the absence of the character for localization, and the pollen parents possessed it either in the homozygous or the heterozygous condition. It was expected, therefore, that each set of seedlings would show either all necrotic-type, or one-half necrotic-type plants. This expectation was realized, for in 8 progenies there were altogether 201 necrotic type and no mottling-type plants, and in 7 progenies in which there were found both types, they were in approximately a 1:1 ratio, 94 seedlings proving to be of the necrotic type and 88 of the mottling type. The fourth generation thus added to the proof previously obtained, that the hereditary factor for localization of virus acts as a simple, dominant Mendelian factor. It also indicated that no incompatibility exists between the factor for localization of tobacco-mosaic virus and certain factors characterizing commercial types of peppers. The plants of this generation resembled commercial varieties in plant and fruit type more nearly than had the first 3 hybrid generations, yet expressed in full the characteristics of the necrotic-type response shown by Tabasco pepper and by plants of the earlier hybrid generations.[3]

*Capsicum frutescens var. Golden Dawn* × *var. minimum.*—A study was made of a second hybrid, *Capsicum frutescens* var. Golden Dawn × var. *minimum*, in which more known genetic factors were concerned than had been the case with the Tabasco × Ruby King hybrid. *C. frutescens* var. Golden Dawn bears white flowers, green immature fruits, pendant, yellow, mild-flavored mature fruits with blunt apices, and is characterized by green cotyledons and hairy stem not branched at base. *C. frutescens* var. *minimum* has purple flowers, blackish-purple immature fruits, erect, red, pungent

[3] Subsequent back-crosses to commercial varieties have yielded individual plants bearing pendant, blunt-nosed, thick-walled, mild-flavored fruits as large as 95 x 50 mm., and reacting to leaf inoculation with tobacco-mosaic virus by formation of necrotic lesions, localization of virus, abscission of inoculated leaves, and subsequent healthy growth, just as in the original Tabasco pepper.

mature fruits with pointed apices, purplish cotyledons, and smooth stem branching from base.

All plants of the first generation of *Capsicum frutescens* var. Golden Dawn × var. *minimum* showed purple-edged white flowers, immature fruits tinged with purple, partly pendant, red, pungent, mature fruits with pointed apices, green cotyledons, and nearly smooth stem branching at base. Twenty-six first-generation hybrid plants were grown. They were all inoculated with tobacco-mosaic virus, and all responded like the necrotic-type parent, showing necrotic primary lesions, abscission of inoculated leaves, and subsequent healthy growth.

In $F_2$ plants grown from seed of selfed $F_1$ plants an approximate 3:1 ratio between necrotic-type and mottling-type individuals was found, as expected; in a set of 1277 the ratio was 2.93:1. A group of 107 plants was examined for evidence of linkage between the factor for virus localization (*L*) or its recessive allelomorph (*l*) and several previously known genes[4] of the pepper: pungent (M) vs. mild (m) fruit, pendant (P) vs. erect (p) fruit, red (R) vs. yellow (r) mature fruit, purple (A) vs. white (a) flower, smooth (H) vs. hairy (h) stems, and pointed (D) vs. blunt-nosed (d) fruits. No evidence was found suggesting linkage between the gene for localization of tobacco-mosaic virus and any of the others. This is not surprising in view of the fact that there are 12 chromosomes in *Capsicum frutescens*.

Among 109 $F_2$ plants obtained as a result of the back-cross *Capsicum frutescens* var. Golden Dawn × (var. Golden Dawn × var. *minimum*), 54 proved to be of necrotic type and 55 of mottling type. No evidence was found for linkage between the gene for virus localization and any other.

Similar observations on a set of 113 plants derived by selfing a plant from the earlier Tabasco hybrid stocks gave no evidence of linkage between the factor for green (G) vs. pale (g) immature fruit and that for virus localization.

Data showing the distribution of the localizing gene and 7 other genes are summarized in table 1.

### Response to Infection with Other Strains of Tobacco-mosaic Virus

It was found possible to test the response of individual pepper plants to tobacco-mosaic virus without producing systemic infection even of mottling-type plants. This was done by picking a leaf from each plant to be tested, inserting its petiole into a test-tube full of water, and then inoculating the

[4] The genes for purple flower and pendant, red, and pointed fruit are referred to in accordance with the usage of Deshpande, R. B., Studies in Indian chillies. (3) The inheritance of some characters in *Capsicum annuum* L. Indian Jour. Agr. Sci. 3: 219–300. 1933.

TABLE 1.—*Absence of linkage between the virus-localizing factor (L) and certain other genetic factors in Capsicum frutescens*

| | Factors involved; L (localizing) and X | Observed ratios | Total number of observed plants |
|---|---|---|---|
| | X equals: | LX : Lx : lX : lx | |
| $F_2$ from selfed $F_1$; ratios expected in absence of linkage, 9:3:3:1 | R (red) | 49 : 22 : 26 : 9 | 106 |
| | M (pungent) | 55 : 15 : 21 : 14 | 105 |
| | A (purple) | 53 : 19 : 28 : 7 | 107 |
| | P (pendant) | 54 : 18 : 23 : 11 | 106 |
| | H (smooth) | 52 : 19 : 27 : 8 | 106 |
| | D (pointed) | 56 : 15 : 30 : 5 | 106 |
| | G (green) | 58 : 28 : 21 : 6 | 113 |
| Back cross; ratios expected in absence of linkage, 1:1:1:1 | R (red) | 31 : 23 : 23 : 31 | 108 |
| | M (pungent) | 29 : 24 : 34 : 20 | 107 |
| | A (purple) | 27 : 27 : 33 : 22 | 109 |
| | H (smooth) | 27 : 26 : 33 : 22 | 108 |

detached leaf. Leaves from necrotic-type plants responded with necrosis as they would if they were still attached to the plants. Leaves from mottling-type plants showed no necrosis, sometimes appearing unaffected by the inoculation, sometimes showing slight chlorosis in primary lesions, or, if yellowing from age, slight chlorophyll retention, as would be expected if the leaves were still attached to the plants.

This detached-leaf test allowed single plants to be tested for possible necrotic response to more than one strain of virus. Successive sets of leaves from plants of known response to the distorting strain of tobacco-mosaic virus were inoculated with the mottling and masked strains of tobacco-mosaic virus described elsewhere (5), and with virus of aucuba mosaic of tomato, propagated in *Nicotiana tabacum*. The set of 109 plants of *Capsicum frutescens* var. Golden Dawn × (var. Golden Dawn × var. *minimum*) already tested by the detached-leaf method for response to infection with the distorting strain of tobacco-mosaic virus, was used to supply leaves for tests with the other virus strains. Two of the plants of the set were not at the time available for this test, and among the 107 tested plants, leaves of which had been inoculated with the distorting strain of tobacco-mosaic virus, the ratio was 52 necrotic (heterozygous for localizing gene, *i.e., Ll* plants) to 55 nonnecrotic (*ll* plants). The same 52 plants that responded with necrosis on leaves inoculated with the distorting strain of tobacco-mosaic virus also showed necrosis of the same sort on additional leaves inoculated with the two mild strains, the mottling and masked strains of tobacco-mosaic virus.

The remaining 55 plants showed no necrosis after inoculation with these strains. Inoculation of a set of leaves with virus of aucuba mosaic of tomato in juice of Turkish tobacco plants induced typical necrotic primary lesions with pale brown center and dark brown periphery on the 52 necrotic-type plants of the set, but the formation of brown, imperfectly necrotic lesions on the leaves of all the mottling-type plants also.

When inoculated leaves were not detached from plants the response to these virus strains was as follows. Inoculation with the masked strain induced slight stunting and mild mottling in *ll* plants, necrosis and leaf abscission, with complete loss of virus in *LL* plants. Inoculation with the mottling strain induced somewhat similar stunting and mottling in *ll* plants, and the usual necrosis, abscission, and loss of virus in *LL* plants. Inoculation with the virus of aucuba mosaic of tomato induced severe stunting, blanching of young leaves, and occasional systemic necrosis in some *ll* plants, but imperfectly necrotic primary lesions, abscission of inoculated leaves, and loss of virus in others; all *LL* plants showed typical primary necrosis, leaf abscission, and loss of virus.

The similarity of necrotic primary lesions in all necrotic-type plants when infected with these 4 strains of tobacco-mosaic virus suggests that the factor that determines virus localization and necrotic response to infection with the common type of tobacco-mosaic virus may act similarly in response to infections with many, possibly all, strains of this virus.

### NICOTIANA

The simple Mendelian type of inheritance of the character for virus localization in Capsicum L. suggested the possibility that the same or a similar character might occur in other plants that show necrotic lesions when infected with tobacco-mosaic virus. Eight crosses were made between necrotic-type and mottling-type species and varieties of Nicotiana. In addition, 2 crosses between pairs of necrotic-type species and 1 cross between a pair of mottling-type species were tested. The number of plants involved was not large, but the response in hybrid progenies was definite, and tended to confirm the results obtained with Capsicum. Details of these experiments follow.

*Nicotiana tabacum (mottling) × glutinosa (necrotic).* One Nicotiana cross between a mottling-type and a necrotic-type species was made by Allard (1) in 1914. This was the hybrid *N. tabacum × glutinosa,* the latter species being known to him not as *N. glutinosa,* but as *N. viscosum.* Allard found that the necrotic response of the pollen parent was transmitted to the first-generation hybrid. In view of the fact that the necrotic response of *N. glutinosa* had not been studied intensively at that time, it seemed desirable to obtain the hybrid again in order to determine whether the first-

generation plants reproduced the necrotic response of the pollen parent fully, or in modified form.

The variety *purpurea* of the mottling-type species *Nicotiana tabacum* was chosen as seed parent and pollen from *N. glutinosa* was applied to it. The seeds thus obtained germinated well, and the seedlings which grew from them strongly resembled *N. tabacum* in leaf character and flower color. Inoculation of the leaves with tobacco-mosaic virus was followed, however, not by mottling, but by the appearance of necrotic spots closely resembling those produced in *N. glutinosa*. This response was given by all of 319 inoculated $F_1$ plants. To this extent the necrotic response appeared to be a dominant character, as was the case with the similar necrotic response in the pepper. Unlike the pepper hybrids, however, this Nicotiana hybrid was completely sterile, setting no seed during an entire year. For this reason the behavior of the factor responsible for the necrotic response has not been observed in a second or later generation. A photograph of the necrotic primary lesions in the first-generation hybrid is shown in figure 3, A.

*Nicotiana bigelovii var. quadrivalvis (yellowing) × glutinosa (necrotic).* —*N. bigelovii* Wats. var. *quadrivalvis* Pursh. is more severely affected than *N. tabacum* by infection with tobacco-mosaic virus. A prolonged period of stunting of the plant and yellowing of all young leaves precedes formation of mottled and distorted new leaves. The hybrid *N. bigelovii* var. *quadrivalvis × glutinosa* was tested to determine whether it would respond as had the hybrid *N. tabacum × glutinosa*. Necrotic primary lesions like those characteristic of *N. glutinosa* appeared on all of 135 inoculated plants of the first-generation hybrid. One hundred similar plants, not inoculated with virus, were grown through an entire summer. Their profuse blossoming did not result in seed formation. Because of this, no evidence was obtained for behavior of the factor for necrosis in later generations. It was evident, however, that in the first generation the necrotic response of *N. glutinosa* was fully dominant over the yellowing and mottling response of *N. bigelovii* var. *quadrivalvis*, as it had been over the mottling response of *N. tabacum* var. *purpurea*.

*Nicotiana bigelovii var. quadrivalvis (yellowing) × sanderae (necrotic).* —Similar evidence indicating dominance of the factor for necrosis in *N. sanderae* Sander was obtained from a single plant of *N. bigelovii* var. *quadrivalvis* (yellowing) × *sanderae* (necrotic), which was secured upon attempting to germinate seed formed as a result of the appropriate pollination. The hybrid bore large reddish-purple blossoms, but did not form seeds; its leaves resembled those of *N. bigelovii*, but were of coarser texture. Upon inoculation with tobacco-mosaic virus, it responded by formation of necrotic lesions resembling those of *N. sanderae* (Fig. 3, B).

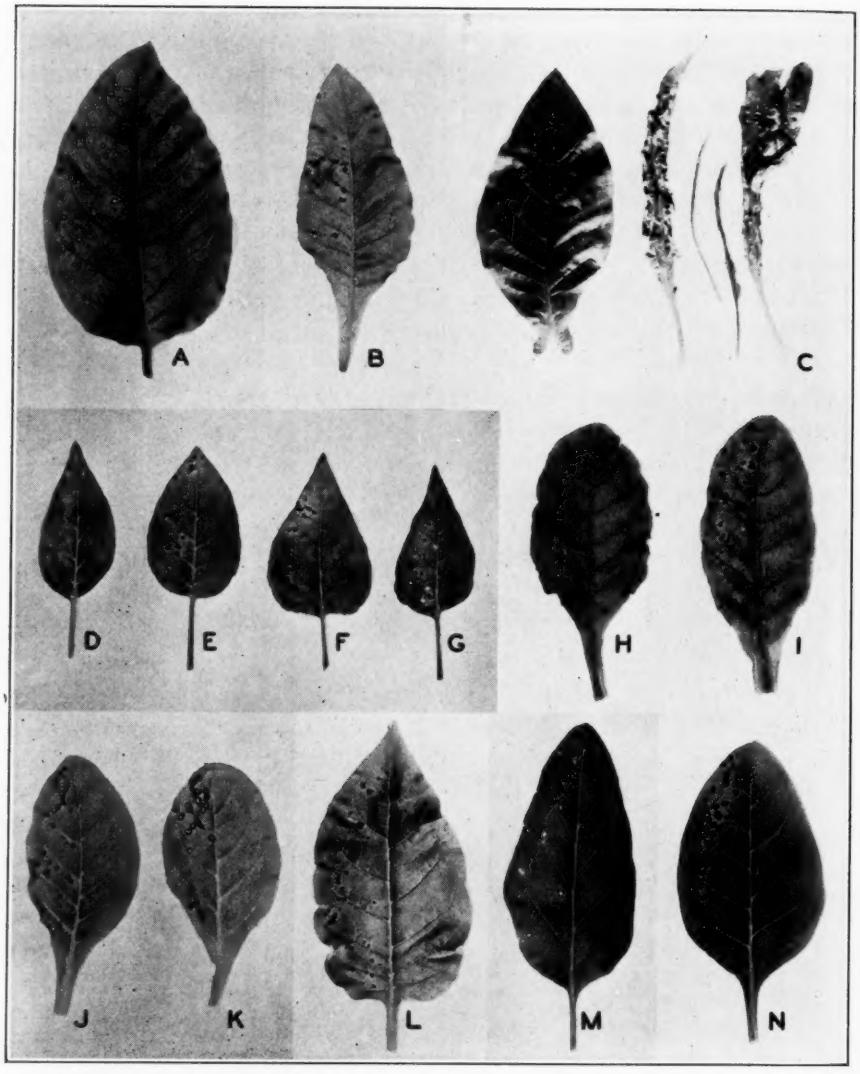


FIG. 3. A and B. Inoculated leaves. A. *Nicotiana tabacum* var. *purpurea* × *glutinosa*. B. *N. bigelovii* var. *quadrivalvis* × *sanderae*. C. Healthy leaf and 4 systemically infected, mottled, and distorted leaves of *N. bigelovii* var. *quadrivalvis* × *tabacum* var. *purpurea*, showing enations. D–N. Inoculated leaves. D. *N. caudigera*. E. *N. acuminata* × *caudigera*. F. *N. acuminata*. G. *N. caudigera* × *acuminata*. H. *N. sanderae* (mottling) × *langsdorffii*, recessive individual of $F_2$. I. *N. sanderae* (mottling) × *langsdorffii*, necrotic-type individual of $F_2$. J. *N. langsdorffii* × *sanderae* (necrotic). K. *N. sanderae* (necrotic) × *langsdorffii*. L. *N. glutinosa* × *langsdorffii*. M. *N. paniculata* × *rustica* var. Winnebago. N. *N. glauca* × *langsdorffii*.

*Nicotiana caudigera* (*mottling*) × *acuminata* (*necrotic*).—Although some Nicotiana hybrids formed no viable seeds, it was found possible to obtain several fertile hybrids, in which the responses of second-generation plants could be studied. One instance was that of the fertile hybrid *N. caudigera* Phil. × *acuminata* Hook. *N. caudigera* shows intense yellowing and stunting, followed by the production of distorted, mottled leaves after inoculation with tobacco-mosaic virus. *N. acuminata* shows no mottling, but only brown, necrotic primary lesions as a result of infection. The $F_1$ hybrid, *N. caudigera* × *acuminata*, showed necrosis like that of the pollen parent in all of 135 inoculated plants. The first-generation hybrid set seed abundantly. Seed from a single plant of this first generation was planted and germinated well. The seedlings were not uniform in appearance, some growing rapidly, others slowly. This was not unexpected, since *N. acuminata* had been observed to grow much more rapidly than *N. caudigera*. Altogether, 228 plants of the second hybrid generation were grown and inoculated with tobacco-mosaic virus; 193 responded with necrosis like that of *N. acuminata*, 35 with chlorosis like that of *N. caudigera*. The ratio of necrotic to chlorotic plants was therefore 5.5 : 1. The reciprocal $F_1$ hybrid, *N. acuminata* × *caudigera*, also proved to be of the necrotic type. Inoculated leaves of the two parent species and of reciprocal hybrids of the first generation are shown in figure 3, D to G.

The facts that the first-generation hybrids all showed a necrotic response, and that the second-generation plants were divided into two groups, giving respectively necrotic and chlorotic responses, seem to indicate that in Nicotiana, as in Capsicum, a dominant factor determines the necrotic response to infection with tobacco-mosaic virus, the corresponding recessive allowing systemic mottling. The reason for the excess of necrotic-type plants beyond the expected 3 : 1 ratio in the second generation is not known.

*Nicotiana sanderae* (*mottling*) × *langsdorffii* (*necrotic*).—In commercial lots of seed of the ornamental, red-flowering *N. sanderae*, which originated many years ago from the cross *N. alata* Link & Otto × *forgetiana* Sand., the factor determining a necrotic response is typically present, but not invariably so. It is not unusual to obtain plants of *N. sanderae* that mottle when infected with tobacco-mosaic virus. A mottling-type plant of *N. sanderae* var. Crimson King was used as seed parent in crosses with *N. langsdorffii* Schrank, and *N. sanderae* (necrotic), both crosses producing fertile hybrids.

*N. langsdorffii* is not variable in response, as is *N. sanderae*, but responds invariably with necrosis. Only 13 first-generation hybrids were obtained of *N. sanderae* (mottling) × *langsdorffii*. Twelve of these responded to infection with tobacco-mosaic virus with necrosis similar to that shown by *N. langsdorffii*. The remaining plant responded with yellowing primary

lesions, and later mottling, but showed evidence of being an actual hybrid by the form of its blossoms, which resembled those of *N. langsdorffii,* as did those of the other $F_1$ plants. Seed was obtained from the single mottling $F_1$ plant and from several of the necrotic-type $F_1$ plants.

A group of 57 plants from seeds of the one exceptional mottling $F_1$ plant showed typical localized necrosis in 10, which subsequently grew as healthy plants, mottling in 24, and systemic necrosis resulting in death in 23 individuals. The reason for failure of dominance of the necrotic response in the $F_1$ plant is not known. Some additional genetic factor may have operated in this exceptional case, preventing the necrotic response in the $F_1$ plant in spite of the presence of the factor for necrosis. The factor for necrosis was shown to have been present by its reappearance in the $F_2$ generation.

Seedlings derived from one of the 11 necrotic-type $F_1$ plants, upon inoculation with tobacco-mosaic virus, showed a necrotic-type response in 134 individuals, and a mottling-type response in the remaining 45, giving the ratio 2.98 : 1 (Fig. 3, H and I). The factor for necrosis in this instance appeared to be dominant in the usual sense.

*Nicotiana sanderae (mottling) × sanderae (necrotic).*—The same mottling-type plant of *N. sanderae* var. Crimson King used in the cross with *N. langsdorffii,* was used as seed parent in a cross with a necrotic-type *N. sanderae* plant. The $F_1$ hybrid, *N. sanderae* (mottling) × *sanderae* (necrotic) responded to inoculation with tobacco-mosaic virus by showing necrosis in all of 84 inoculated plants. In the second generation, 249 plants were inoculated; 185 responded with necrosis like that of the original pollen parent and of the $F_1$ plants, and 64 responded with chlorosis like that of the seed parent (ratio 2.88 : 1). The necrotic response in this cross appeared fully dominant, and no evidence of interference by other genetic factors was observed.

*N. paniculata (mottling) × rustica (necrotic).* Two other hybrids were produced between species showing necrosis and those not showing necrosis as a result of infection with tobacco-mosaic virus. They were *N. paniculata L.* × *rustica* L. and *N. glauca* R. Grah. × *langsdorffii;* these were studied because of responses of the seed parents, one of which produces enations and the other of which is symptomless, or nearly so, when infected.

*N. paniculata* responds to infection with tobacco-mosaic virus by mottling, and by forming enations or tissue outgrowths (6). *N. rustica* responds with systemic necrosis when young, and by localized necrosis when older. Twenty-one plants of the first-generation hybrid, *N. paniculata* × *rustica* var. *jamaicensis,* all responded with necrosis closely resembling that of the pollen parent; the necrosis was systemic in young plants, but localized in inoculated leaves of older ones. *N. rustica* var. Winnebago shows necrotic

lesions characterized by the formation of more dark brown pigment at the periphery of the lesion than occurs in the variety *jamaicensis*. Eleven plants of the hybrid *N. paniculata* × *rustica* var. Winnebago were inoculated, and 10 responded with lesions surrounded with dark brown pigment of the type of the pollen parent (Fig. 3, M). The 11th plant was atypical in appearance, being round-leaved and dwarfed; it also responded with necrosis, but not with dark brown pigment. The difference in type of necrotic lesion found in the two *N. rustica* varieties thus appeared, with the one exception noted, in the first generation hybrids. No sign of the tendency of *N. paniculata* to form enations appeared in any of the hybrids. Although only 32 $F_1$ plants were grown, they agreed with other crosses in producing all necrotic-type plants from mottling and necrotic-type parents.

*Nicotiana glauca* (*nearly symptomless*) × *langsdorffii* (*necrotic*). *N. glauca* mottles faintly if infected with tobacco-mosaic virus when young. In older plants the virus multiplies well in inoculated leaves, moves toward the top of the plant erratically, and when present in the younger leaves sometimes causes traces of mottling, and sometimes no symptoms. *N. langsdorffii*, as stated before, shows a necrotic response with production of black-brown pigment at the periphery of primary lesions. A set of plants of the first generation of *N. glauca* × *langsdorffii* was grown and 198 plants were inoculated; all responded with necrosis (Fig. 3, N) resembling that characteristic of *N. langsdorffii*.

*Nicotiana langsdorffii (necrotic)* × *sanderae (necrotic)*. If the apparently similar genetic factors for necrosis were fundamentally unlike in some of the species already mentioned, combining such dominant factors in a single $F_1$ plant might result in a modified response. Only two pairs of necrotic-type species were tested. The first of these was *N. langsdorffii* × *sanderae* (necrotic). The response of the first-generation hybrid did not differ from that of the parent species; necrotic lesions surrounded by zones of blackish brown pigment (Fig. 3, J) were produced in all of 162 $F_1$ plants. There appeared to be no additive effect upon combining dominant factors from the two parents in individual hybrid plants. The reciprocal hybrid, *N. sanderae* (necrotic) × *langsdorffii* (Fig. 3, K), was also grown and 92 plants, after being inoculated with tobacco-mosaic virus, all showed a necrotic response like that of the parents and of *N. langsdorffii* × *sanderae*. If the genetic factors in the two parents were similar in nature, but not located in corresponding chromosomes, some plants of a second generation should fail to receive the factor for necrotic response from either parent, since there would be independent segregation. In a second generation grown from seeds of selfed *N. sanderae* × *langsdorffii* plants, all of 255 inoculated plants showed necrotic primary lesions of the type shown in the first hybrid generation. The dominant genetic factors determining the necrotic response

in these two Nicotiana species acted as though identical, giving no obvious additive effect when combined, and appearing in all $F_2$ plants.

*Nicotiana glutinosa (necrotic) × langsdorffii (necrotic).* The second combination of two necrotic-type species was that of *N. glutinosa* and *N. langsdorffii.* Only a single plant of the hybrid *N. glutinosa × langsdorffii* grew from seed obtained as a result of appropriate pollination. It was intermediate in appearance between the unlike parent species and proved self-sterile and sterile to *N. langsdorffii* pollen. Upon inoculation with tobacco-mosaic virus it formed necrotic primary lesions (Fig. 3, L) closely resembling those of *N. glutinosa,* but surrounded by a somewhat darker brown peripheral zone, only a little less blackish than that characteristic of *N. langsdorffii.*

No evidence was found in the behavior of the two necrotic-type combinations, *N. sanderae* (necrotic) × *langsdorffii* and *N. glutinosa × langsdorffii,* to indicate that the dominant factors determining necrotic response are different in the species *N. sanderae, N. langsdorffii,* and *N. glutinosa.*

*Nicotiana bigelovii var. quadrivalvis (yellowing) × tabacum (mottling).* —Since the hybrids of *N. tabacum* and *N. quadrivalvis* with *N. glutinosa* had resulted in $F_1$ plants of necrotic type, it was desirable to test the hybrid *N. bigelovii* var. *quadrivalvis × tabacum,* although it seemed unlikely that there would be production of necrotic-type plants without the use of a necrotic-type parent. The first-generation hybrid *N. bigelovii* var. *quadrivalvis × tabacum* var. *purpurea* was produced and about 100 plants were grown to blossoming, sets of the plants being inoculated at intervals with tobacco-mosaic virus. The hybrid stock responded to infection by stunting, distortion, and mottling. No necrosis appeared at any time. Outgrowths of tissue (enations) from the lower surface of mottled leaves were numerous in the infected plants (Fig. 3, C), although these had been grown in shade, a condition not suitable for formation of enations on plants of *N. paniculata,* as shown by Jensen (6).

Neither the failure to secure plants of a second generation from some Nicotiana hybrids, nor the small numbers of $F_1$ plants obtained from certain crosses need obscure the result of the study of this genus as a whole. No deviation from the parental responses was found when two necrotic-type species were crossed with each other. A summary of tested plants in eight crosses between mottling-type and necrotic-type plants is shown in table 2. Altogether, 917 $F_1$ plants were inoculated with tobacco-mosaic virus; all but one individual responded with necrosis. The single exception had as one parent the rather variable horticultural form known as *N. sanderae;* some interfering genetic character or combination of characters may have been responsible for the anomalous result. From 3 of the 8 crosses a second generation was secured by selfing plants of the $F_1$ generation. A total of

TABLE 2.—*Summary of tests showing behavior of factor for necrosis in Nicotiana hybrids*

| | First hybrid generation | | Second hybrid generation | | |
|---|---|---|---|---|---|
| | Necrotic type plants | Mottling type plants | Necrotic type plants | Mottling type plants | Ratios |
| *Nicotiana tabacum* × *glutinosa* | 319 | 0 | ... | ... | ... |
| *Nicotiana bigelovii* var. *quadrivalvis* × *glutinosa* | 135 | 0 | ... | ... | ... |
| *Nicotiana bigelovii* var. *quadrivalvis* × *sanderae* (necrotic) | 1 | 0 | ... | ... | ... |
| *Nicotiana caudigera* × *acuminata* | 135 | 0 | 193 | 35 | 5.5:1 |
| *Nicotiana sanderae* (mottling) × *langsdorffii* | 12 | 1 | 134 | 45 | 3.0:1 |
| *Nicotiana sanderae* (mottling) × *sanderae* (necrotic) | 84 | 0 | 185 | 64 | 2.9:1 |
| *Nicotiana paniculata* × *rustica* | 32 | 0 | ... | ... | ... |
| *Nicotiana glauca* × *langsdorffii* | 198 | 0 | ... | ... | ... |
| Totals | 916 | 1 | 512 | 144 | 3.6:1 |

656 $F_2$ plants were inoculated. A considerable excess of necrotic-type plants occurred in the $F_2$ generation of *N. caudigera* × *acuminata* (193 necrotic-type; 35 mottling-type); the other $F_2$ sets gave ratios of 3.0:1 and 2.9:1 respectively. The conclusion seems warranted that in the genus Nicotiana, as in Capsicum, a necrotic response to infection with tobacco-mosaic virus acts as a typically dominant character in certain crosses between necrotic-type and mottling-type forms.

### SOLANUM MELONGENA

In the genera Capsicum and Nicotiana, factors for necrotic response to inoculation with tobacco-mosaic virus behaved similarly, being in both cases dominant over factors for mottling response. In the genus Solanum, the species *S. melongena* L. (eggplant) shows both necrotic and mottling types of response among its varieties. It seemed probable that in this genus also the factor for necrosis might be dominant over that for mottling. This was not found to be entirely true, however.

*Solanum melongena* var. Peking Green mottles inconspicuously if infected with tobacco-mosaic virus when young; infected plants tend to become symptomless with age. The variety Hangchow Long is symptomless regardless of age at time of inoculation, but allows virus to increase in its tissues and to spread systemically. The variety Black Beauty is killed by systemic necrosis if infected as a small seedling, but localizes the virus completely and shows necrosis only at the site of inoculation on leaves

of older plants. The mottling-type variety Peking Green and the symptomless variety Hangchow Long were both pollinated from the necrotic-type variety Black Beauty.

*Solanum melongena var. Peking Green × var. Black Beauty.*—The first-generation hybrid *S. melongena* var. Peking Green × var. Black Beauty responded to infection with tobacco-mosaic virus in all of 103 inoculated plants by production of faintly yellowish primary lesions. Around most of these lesions, however, flecks of necrosis later appeared, sometimes developing as thin, light brown, necrotic, circular or wavy lines. A faint systemic mottling later developed, again marked by occasional thin, light brown, necrotic concentric rings and other necrotic markings outlining infected areas. The leaves affected by the systemic spread of the virus were characterized by not being so flat as the corresponding leaves of healthy plants; cup-like depressions and slightly rugose leaf surface seemed to be caused by irregularities in rate of expansion of the lamina. The first-generation hybrids tended toward a symptomless condition in later growth. It was evident that the $F_1$ plants did not respond like either parent.

A second hybrid generation was grown from seed of several plants of the first-generation hybrid. Among the second-generation plants the solidly necrotic, dark brown primary lesions and similar secondary lesions in young plants characteristic of the necrotic-type parent, Black Beauty eggplant, reappeared in some plants. A considerable part of the remaining individuals showed flecks of ring-necrosis such as had characterized infected $F_1$ plants. It seemed probable that in this Solanum hybrid the necrotic response was not wholly dominant, although a single pair of allelomorphs might possibly account for the responses. The heterozygous plants were irregular in time of formation of traces of necrosis. For this reason attention was directed to observing the ratio of fully necrotic $F_2$ plants to those showing traces of necrosis or none at all. This ratio, 132 : 333, amounted to about 1 : 2.5, and so might be considered to support to some extent the hypothesis that a single Mendelian factor for necrosis existed in *Solanum melongena*, as in *Capsicum frutescens*, and some Nicotiana species, although in the case of *S. melongena* dominance appeared imperfect, and the heterozygotes could not be distinguished readily from the non-necrotic segregates.

*Solanum melongena var. Hangchow Long × var. Black Beauty.*—The first-generation hybrid *S. melongena* var. Hangchow Long × var. Black Beauty responded to infection with tobacco-mosaic virus in all of 150 inoculated plants by production of yellowish primary lesions peripherally marked with traces of necrosis and by necrotic flecks on younger leaves. The symptoms were similar to those on the $F_1$ Peking Green × Black Beauty eggplant. In the second generation, a fully necrotic response, like that of Black Beauty eggplant, was noted in 199 of 743 inoculated plants. This gives the ratio of fully-necrotic plants to those not fully necrotic or show-

ing no necrosis as about 1:2.7, a slightly closer approach to a 1:3 ratio than had been obtained with the Peking Green × Black Beauty hybrids.

This second *Solanum melongena* hybrid confirms the conclusion reached from observation of the preceding form, that the necrotic response in this species is not fully dominant, but is probably of the nature of a Mendelian character, as in Nicotiana and Capsicum species.

## DISCUSSION

The characteristic of localizing tobacco-mosaic virus, because of its easy manipulation as an hereditary unit, may be a useful tool in attempting to gain an understanding of the nature of viruses. A plant that possesses the dominant character is able to restrain tobacco-mosaic virus from producing a systemic infection, although without this factor it would be unable to do so. It has been shown in another paper (5) that several distinct strains of tobacco-mosaic virus differ in degree of freedom to spread in systemic infection of young leaves. In an earlier paper (4, p. 341) it was shown that a similar situation was discernible in iodine-stained material of various hosts of the distorting strain of tobacco-mosaic virus. It has been shown by Kunkel (7) that the virus of aucuba mosaic is in many characteristics similar to that of tobacco mosaic, but its restricted to necrotic primary lesions in *N. sylvestris* Spegaz. & Comes, in which tobacco-mosaic virus produces a systemic mottling. It appears that slight differences in strains of host plants, typified by the difference of a single genetic factor in the pepper, and slight differences in strains of virus which are in most respects similar, may both make differences in freedom of virus spread and even the difference between a localized necrotic infection and one of systemic mottling. Physical or chemical characteristics of the virus, as well as of the host plant, are probably concerned in this relationship.

## SUMMARY

Localization of tobacco-mosaic virus in *Capsicum frutescens,* the garden pepper, was found to be determined by a dominant Mendelian factor. In plants possessing this factor the virus increased in tissues at the site of inoculation and caused the appearance of numerous necrotic spots of small size, with early abscission of the inoculated leaf and subsequent healthy growth of the plant to maturity. The recessive allelomorph allowed systemic spread of virus, stunting of plant, mottling and distortion of leaves, and reduced yield of fruit.

Somewhat similar genetic factors, determining necrotic response, were found in a number of Nicotiana species and in *Solanum melongena.*

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# MULTIPLICATION OF THE VIRUSES OF TOBACCO AND AUCUBA MOSAICS IN GROWING EXCISED TOMATO ROOT TIPS

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Stock supplies of plant-disease viruses are at present maintained in several different ways. One common method is to preserve samples of filtered juice from infected plants in a frozen state in a refrigerator. This method is fairly satisfactory for the maintenance of viruses that retain their activity for reasonably long periods of time under such conditions. It is not satisfactory for all viruses, however, since many soon become inactivated, even when frozen.

Another standard method of maintaining stocks is by inoculation of plants kept in greenhouses or in insect-proof cages. Pieces of tissue from such infected plants can be used at any time as a source of inoculum. The virus, under these conditions, is maintained in a state of active multiplication. Greenhouse or caged plants, however, require much space and special attention. Moreover, with plants grown in greenhouses or cages, the possibility of accidental contamination by insect, human, or other agencies is always present. The purity of strains so maintained can, therefore, not be absolutely relied upon. Attempts have been made to grow host plants under isolated, aseptic conditions free from this danger, but the techniques so far developed are rather cumbersome. No simple reliable method of maintaining stocks of plant-disease viruses under conditions of known purity, free from danger of contamination, and in a state of active multiplication, has been developed to date. Such a method would obviously be of value, especially where many different viruses are being studied in the same laboratory.

The active principle of several plant-virus diseases is known to be present in the roots of plants systemically infected with these diseases and to increase in concentration with progress of time after infection. Since it has been shown that root tips of the tomato, a plant that is susceptible to many different virus diseases, are capable of growing apparently indefinitely in an isolated state under controlled environmental conditions (32), it has seemed possible that such roots might prove to be excellent experimental material in which to maintain certain viruses under controlled conditions. Such cultivation of the viruses would depend (1) on the infected roots' growing sufficiently well to maintain cultures, and (2) on the multiplication of virus in the roots. No marked symptoms have

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been observed in roots of diseased plants in spite of the presence and increase of virus in such roots. For this reason it has sometimes been supposed that multiplication of virus does not take place in roots, but that its presence and increase in roots may be due to accumulation of virus produced elsewhere in the plants. The work presented here was planned to test some of these possibilities.

## EXPERIMENTAL

The stem of a rapidly growing plant of Bonny Best tomato carrying a pronounced systemic infection with tobacco-mosaic disease was cut up into segments. These were thoroughly washed and so suspended, by single threads in 3-liter Erlenmeyer flasks containing a little water, that the cuttings did not touch either the flask or the water. The flasks were plugged with cotton and left on a laboratory table. Roots developed on the basal portions of the cuttings. After 11 days, 18 root tips were carefully removed and placed in 125-ml. Erlenmeyer flasks, prepared as previously described (31). Each flask contained 50 ml. of nutrient. The nutrient used was prepared as previously described (32) and contained the following substances in the concentrations indicated:

| | |
|---|---|
| $Ca(NO_3)_2$ | 0.60 millimols |
| $MgSO_4$ | 0.30 " |
| $KNO_3$ | 0.80 " |
| KCl | 0.87 " |
| $KH_2PO_4$ | 0.09 " |
| $Fe_2(SO_4)_3$ | 0.006 " |
| Sucrose | 2% (by weight) |
| Yeast (extract) | 0.01% |

Of the 18 roots cultured, 7 became infected with bacteria and had to be discarded, 1 failed to grow, 2 made poor growth and were discarded, 6 were accidentally infected with fungi, and 2 remained in good condition. Of the 6 contaminated roots, 2 succumbed, while 4 were recovered in an aseptic condition by cutting away and discarding the contaminated portions. There were thus 6 roots from which to build up a diseased clone. At the end of a week the 6 surviving cultures were cut into pieces approximately 10 mm. long; the apices were transferred to fresh flasks together with such subapical pieces as were desired for additional cultures, and the discarded basal portions were tested for presence of virus by crushing and rubbing into leaves of *Nicotiana glutinosa* L. All 6 roots contained virus, the individual roots producing from 22 to about 500 lesions. A single root tip was selected for further study. It served as the parent stock from which all subsequent cultures were derived.

The clone was allowed to grow under controlled laboratory conditions and was subcultured (32) at weekly intervals for a period of 30 weeks. At

the 4th passage it contained 50 cultures. This number was maintained through passages 4, 5, 6 and 7, after which enough roots were discarded to reduce the number to 25. The latter number was maintained through all subsequent passages.

At the end of each of the first 4 passages, each culture was tested for presence of virus by inoculation of plants of *Nicotiana glutinosa*. In all passages thereafter, with the exception of the 20th and 30th, only every 5th culture was tested. The virus was present in each root tested in every passage, and although the titer, as shown by the mean number of lesions obtained per culture, varied quite widely from passage to passage, the observed variations were in general apparently related to differences in host-tissue growth rates brought about by external factors. This relationship is shown in figure 1.

At the end of the 20th and the 30th passages, inoculations were made from cultures Nos. 5, 10, 15, 20, and 25 into plants of *Nicotiana tabacum* L.

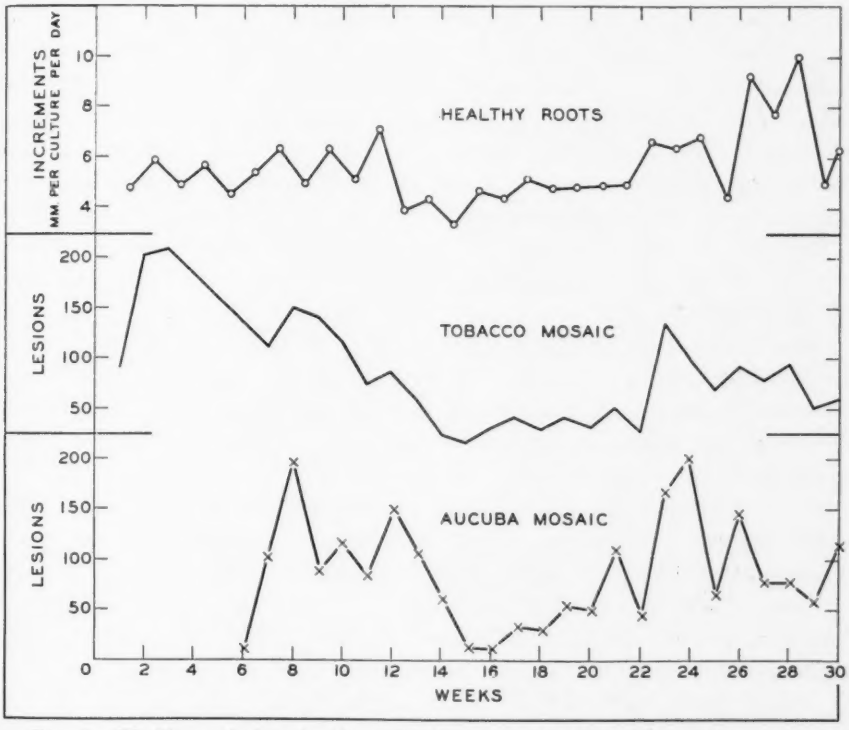


FIG. 1. Weekly variations in titer of tobacco-mosaic virus and aucuba-mosaic virus multiplied in growing isolated root tips and the simultaneous growth rates of healthy roots. The correspondence between the low increment rates from the 12th to the 22nd weeks and the low virus titer obtained in these same weeks is evident.

and *N. sylvestris* Spegaz. and Comes to determine whether the virus was still capable of producing typical symptoms in these plants. At the same time, tissue from each root was inoculated into a leaf of *N. glutinosa* to determine whether virus was present in every culture. At the end of 20 passages, inoculum prepared from every culture except No. 25 induced characteristic local lesions on *N. glutinosa*. Failure in this case was probably due to accidental failure to inoculate the test plant, since the infections produced by other pieces of the same root on plants of *N. tabacum* and *N. sylvestris* were fully systemic and showed no noticeable abnormalities. The results obtained by tests made at the end of 30 passages confirmed those secured by inoculations from roots in the 20th passage. Thirty passages in growing isolated root tips had thus apparently left the virus unchanged in behavior. The theoretical multiplication of host tissue during 30 weeks is estimated to have been of the order $10^{23}$ (32). Since the virus showed no consistent detectable diminution in titer in this time, it is assumed that its increase had kept pace with the multiplication of the host tissue. The virus is being maintained in further subcultures.

Aucuba mosaic was cultivated in the same way through 25 weekly passages in root tips derived from a single cutting of a systemically infected plant. Virus was recovered from these roots by inoculation into *Nicotiana glutinosa* at the end of every passage. At the end of 20 passages, all roots were tested for the presence of virus by inoculation into *N. glutinosa*, and Nos. 5, 10, 15, 20, and 25 were tested for the characteristic symptoms on *N. tabacum* and *N. sylvestris*. The disease produced in these plants was typical in every instance. The infection on *N. sylvestris* showed the characteristic necrotic local lesions without becoming systemic, while on *N. tabacum* it was systemic and characterized by the usual mottling symptoms. The virus had apparently been unaltered by cultivation in isolated tissues through 20 passages, and, although not subsequently tested for symptom expression, was shown to be present after 25 passages. It is being maintained in further subcultures.

Roots infected with each of these viruses were carefully examined for visible symptoms of disease. They were found to be as straight and clean, with as many and as uniform branches, as were the healthy ones. No macroscopic lesions, discolorations, distortion of tissue, change in manner of growth, or other symptoms of disease, could be observed. It is possible that a detailed histological study may disclose the existence of symptoms not visible macroscopically, but it is a remarkable fact that agents that produce such marked distortion, discoloration, and stunting in the aerial parts of the plant as do these two viruses, should leave the roots, even when carrying large amounts of virus, apparently unaffected. Although growth rates are an unreliable criterion for comparison, unless the different cul-

tures to be compared are all derived from the same clonal parent, it is noteworthy that cultures of the clones carrying these viruses grew as rapidly and appeared in as good condition as did those of healthy clones that were being maintained during the time these experiments were in progress.

Experiments were carried out to determine if virus grown in isolated roots escapes into the surrounding medium or can infect healthy roots when added to the medium and whether healthy roots become infected when wounded in the presence of virus or when grown in the same flasks with and in contact with diseased roots. Four rapidly growing cultures infected with tobacco-mosaic disease were selected at the end of the 3rd passage, and a portion of the nutrient from each flask was tested for presence of virus by rubbing into leaves of *Nicotiana glutinosa*. No lesions were produced by the nutrient from any of the 4, while the roots grown in these flasks produced 39, 40, 28, and 253 lesions, respectively. Ten healthy roots, grown in media to which virus was added, did not become infected. In another group of experiments, 12 rapidly growing roots infected with tobacco-mosaic disease, in the 9th passage, were split lengthwise for about 2 mm. Pieces of healthy roots in the 36th passage (clone C, see 32) were then "threaded" through these diseased pieces. Both diseased and healthy fragments grew actively and at the end of 12 days the pieces were removed and tested separately for presence of virus. The originally healthy fragments in no case produced any lesions, while the originally diseased roots produced from 50 to 200 lesions each (average 114 lesions) on comparable test plants. In still another experiment, roots in the 6th passage carrying tobacco-mosaic virus were placed in the bottom of a Petri dish and crushed with a glass spatula. Ten roots of a healthy clone in the 32nd passage were laid in the juice of these diseased tissues and repeatedly wounded with fine insect pins. They were then removed to fresh nutrient and allowed to grow for one week and subcultured and the discarded portions tested for virus. Five of the 10 roots survived and, when tested, produced a few (1 to 47) lesions on leaves of *Nicotiana glutinosa*. At the end of the 2nd passage after inoculation, however, all of the 16 cultures obtained from the 5 surviving roots were virus-free. In a second series of cultures, 10 roots inoculated in the same way were likewise all virus-free in the second passage. Diseased clones of roots have not been established by direct inoculation. They have only been obtained by growing roots from systemically infected plants. Although the experiments performed are insufficient to warrant the conclusion that roots cannot be infected in these ways, it at least appears that the virus does not ordinarily escape from the diseased tissues into the medium, nor is the disease easily transferred to healthy roots by contact with diseased roots or with media to which virus

has been added. Isolated roots are evidently refractory material as far as inoculation by these methods is concerned.

## DISCUSSION

It has been shown that the viruses of aucuba mosaic and tobacco mosaic will multiply actively in growing isolated root tips for periods of at least 25 and 30 weeks, respectively. It may be presumed that they can be so maintained indefinitely. The cultures can be kept under strict physiological control, are entirely free from danger of accidental contamination with other viruses, and require comparatively little space. As a means of maintaining stock cultures of viruses, the method appears to present many advantages over methods previously in use. It would, of course, be dangerous to conclude that all plant-disease viruses can be so cultivated.

The absence of symptoms of disease in the roots is worthy of note. It seems possible that this may have its explanation in the chlorophyll-free character of the host tissue used. The pathological picture ordinarily associated with these diseases, the mottling, distortion, and stunting of the aerial parts of the plant, may be referred to the effects of the disease on the chlorophyll apparatus or to disturbances in metabolism resulting from injury to that apparatus. The roots used in these cultures were apparently free of chlorophyll. Photosynthesis, if occurring at all, was reduced to a very low level, far below that necessary for the maintenance of growth (30), but the requisite product of photosynthesis was supplied to the roots in adequate amounts in the form of sucrose added to the nutrient. The roots were thus freed from dependence on photosynthesis and hence from any injury that might be due to disturbance of this process. The fact that under the conditions described the roots showed no ill effects, although carrying considerable quantities of actively multiplying virus, would seem to suggest that the pathogenicity of these viruses is largely due to specific effects on the process of photosynthesis rather than to injurious effects on tissues in general. The increase of virus in these roots makes the hypothesis, sometimes advanced, that multiplication of virus is dependent on the chlorophyll apparatus, seem untenable.

The comparative ease of controlling the experimental conditions under which the viruses multiplied in these cultures adds considerably to the importance of the method. All of the factors constituting the effective environment, insofar as they are understood, can be controlled (30, 31). The method seems favorable for use in studies on the effects of temperature and light on the growth of viruses. The mineral nutrition may be varied in a much simpler manner than is possible when entire plants are used. The organic nutrition of the host tissues can be more easily controlled than by any other method at present available, since the normal products of

photosynthesis can be varied at will without at the same time altering the external conditions that usually control their production.

The cultivation of the viruses of tobacco mosaic and aucuba mosaic in growing isolated root tips is of interest from another point of view. During the last decade, a number of viruses of animal diseases, notably those of foot-and-mouth disease (Hecke, 16, 17, 18; Maitland and Maitland, 21), herpes (Andrewes, 1, 3; Haagen, 14; Parker, 22; Parker and Nye, 24; Rivers, Haagen and Muckenfuss, 27), myxoma (Haagen, 15; Benjamin and Rivers, 4), vaccinia (Carrel and Rivers, 9; Li and Rivers, 19; Maitland and Laing, 20; Rivers and Ward, 25; Rivers, Haagen, and Muckenfuss, 26, 27, 28; Parker and Nye, 23), variola (Haagen, 13, 14), "Virus III" (Andrewes, 1, 2), fowl-pox (Findlay, 12), vesicular stomatitis (Carrel *et al.*, 10), Rous sarcoma (Carrel, 5, 6, 7; Carrel and Ebeling, 8), pseudorabies (Traub, 29), the common cold (Dochez *et al.*, 11), and others, have been multiplied in cultures to which living tissues have been added. In most cases, however, the media employed have been unsuitable for the indefinite multiplication of the host tissues. The viruses have, therefore, been transferred at each passage to fresh pieces of surviving tissue. In fact, the usual practice has been to use as inoculum not a fragment of infected tissue, but a small amount of supernatant after it has been freed of cells by centrifuging or by filtration. The nutrient used in cultures of plant viruses, while adequate for indefinite multiplication of the host tissues, is also simpler than that commonly used in cultures of animal viruses, since it contains no serum, broth, embryonic juice, tissue extract, or other complex organic constituents, with the exception of a very small amount of yeast extract.

## SUMMARY

A method has been developed for the cultivation of the viruses of tobacco mosaic and aucuba mosaic in isolated root tips grown *in vitro*. The viruses have multiplied actively under controlled, aseptic conditions, in the apparent absence of chlorophyll, and without the production of obvious symptoms. The method provides a simple means of maintaining stocks of these two viruses in a state of active multiplication, yet free from danger of contamination with other viruses.

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# THE MODE OF ENTRANCE OF USTILAGO ZEAE INTO CORN[1]

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## INTRODUCTION

In recent years we have witnessed an awakening to the importance of losses in corn due to common "boil" smut (*Ustilago zeae* (Beckm.) Unger). It is conceded that this ever-present disease annually destroys about two per cent of the corn crop in the United States, and this two per cent amounts to approximately 55,000,000 bushels. There appears to be no practicable method of control other than the breeding of resistant varieties. Therefore, since 1920, much time and energy have been devoted to a study of the disease and the development of resistant varieties of corn.

Stakman and his coworkers (4, 8, 9) have shown that *Ustilago zeae* is heterothallic, that some monosporidial or gametic lines are extremely variable, and that in all probability races distinctly different in pathogenicity exist in nature. They have presented evidence that a given line of corn may be resistant to certain combinations of monosporidial lines and susceptible to other combinations. Eddins (5) found that 12 multisporidial cultures from different localities in Iowa differed from each other in their pathogenicity on inbred lines of corn.

Plant breeders have isolated selfed lines of corn highly resistant to smut and have combined certain sweet-corn lines to produce fairly resistant, high-yielding, sweet-corn hybrids. They have met difficulties, however, in the synthesis of highly resistant, high-yielding varieties of field corn, and it was these difficulties which made clear the need for a general study of the factors influencing the development of smut and the nature of resistance of corn to smut. As the method of entrance of *Ustilago zeae* has been studied but little since the time of Brefeld, it appeared that a study of penetration was a necessary step to clear the way for studies on the nature of resistance.

Julius Kühn was, according to Brefeld (1), the first to inoculate corn with *Ustilago zeae* and observe the entrance of the germ tubes. Kühn reported his observations on one infected plant in a single paragraph in connection with a report of more detailed studies on other smuts. The broad

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scope of Brefeld's studies on *U. zeae* was perhaps partly due to the many distinct differences between corn smut and closely related smuts of small grains and sorghum. The appearance of galls in all rapidly developing portions of the plant, the sharp localization of the mycelium, and the failure of seedling infection were all in sharp contrast to what he had found in other smuts. Brefeld (2, 3) observed that only tender tissues could be penetrated, that infection is purely local, and that only infections in growing tissues result in galls. He figured the direct penetration of the leaf epidermis by conidial germ tubes. In his discussions of the dissemination of inoculum he stressed "air conidia," or "sporidia" as we now loosely term them, apparently considering that chlamydospores serve merely for the overwintering of the fungus and germinate only by the production of promycelia, from which the sporidia or conidia are budded.

## MATERIALS AND METHODS

Preliminary studies were made on standard varieties of corn inoculated with combinations of monosporidial lines known to produce galls. At first, Brefeld's method of studying free-hand sections stained with eosin or similar dyes was tried, but with little success. Next, attempts were made to strip the epidermis from the leaves, but again with little success. Then the lactic acid-cotton blue method was tried on leaf-spiral material. This method was superior to the first two, but was not entirely satisfactory because not enough light passess through the entire thickness of leaf for the use of the fairly high magnification necessary for studying penetration by the very small smut hyphae. We therefore resorted to the paraffin method of histological study.

Plants 6 to 15 inches tall were inoculated in the greenhouse, most commonly by the hypodermic-syringe method, using single monosporidial lines, combinations of lines known to produce galls, and chlamydospores in water suspension. Standard varieties of field and sweet corn and selfed lines producing different reactions were used. Material from the leaf-spirals of inoculated plants was killed and fixed on the 2nd, 3rd, 4th, 5th, 6th, and, in a few cases, the 8th days after inoculation. Later, nodal buds or portions of tassel and stalk were killed and fixed as soon as the first symptoms of smut appeared. Material also was killed and fixed from plants inoculated by 2 more nearly natural but less effective methods, namely, (1) spraying the entire plant with a sporidial suspension, and (2) dropping the sporidial suspension into the leaf-spiral.

In the spring of 1932 selected portions were killed and fixed from plants developing the first symptoms of smut under natural conditions. On this material it was much more difficult to find inoculum than on plants inoculated in the greenhouse, and on the latter the loss of time and trouble was

great because the water suspensions of inoculum spread too freely between the rolled leaves. During the summer a method of inoculation that made it much easier to find fairly heavily inoculated surfaces suggested itself. The hypodermic syringe was used to force dry chlamydospores into the "hearts" of the plants. The syringe was set with the plunger half drawn and the needle was dipped into a test tube of dry chlamydospores. Then the needle tip was driven to the "heart" of a plant and the plunger was pressed, forcing the spores from the tip of the needle by a puff of air. This method sometimes scattered chlamydospores a distance of one centimeter from the point of puncture in the tender leaf-spiral tissues, but it left the spores sufficiently massed to enable selection of material with abundant inoculum. Within 48 hours after introduction by this method the spores had germinated well.

Various killing and fixing agents were tried, but Gilson's fluid and Chamberlain's special chromic-acetic-osmic mixture were used most commonly. Numerous stains and combinations of stains also were tried, but Stoughton's (10) Thionin and Orange G combination was perhaps the most satisfactory for differentiating the fungus and host tissues.

During August and September of 1932 considerable time was given to the study of naturally and artificially inoculated silks of various varieties and selfed lines, some of which are very susceptible to ear smut. For this study vital staining with Thionin and immediate examination was depended upon because of the difficulties involved when silks are processed by the paraffin method. On the assumption that infection through the silk might be dependent on fertilization or pollen-tube growth, care was taken in the later trials in this series to supply the inoculated silks with fresh pollen, since shoots in the first stages of silking were cut from plants in the field and placed in laboratory moist chambers. Care also was taken to allow slow drying in some cases, after the spores had started to germinate, for it is known that the thickness of the film of moisture is important in penetration by certain fungi.

## RESULTS

We have failed to find a single case of peneration of silks by *Ustilago zeae*, although Brefeld (3) reported that infection of the silk tip is easily observed. However, the common observation of single smut kernels deep in otherwise healthy ears, circumstantial evidence though it is, forces us to consider that the silks may be an avenue of infection, despite the fact that many negative tests were made with varied materials and under varied conditions.

Regarding penetration of leaf tissues, the writer's observations are in fair accord with the statements made by Brefeld 45 years ago. The smut

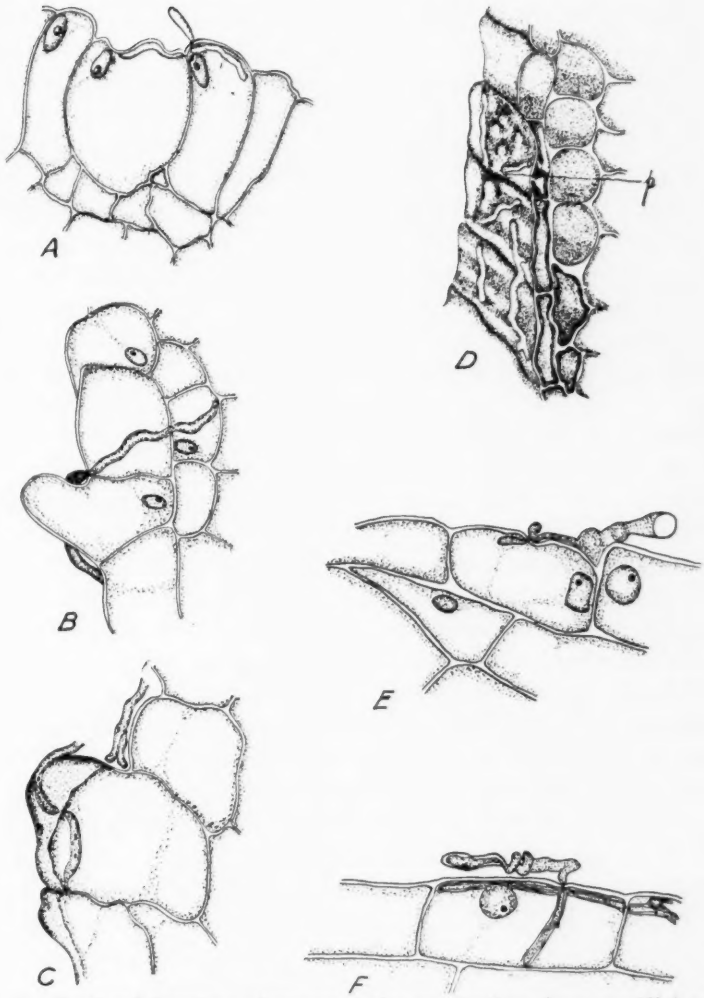


FIG. 1. Freehand drawings of representative penetrations from sporidial germ tubes of *Ustilago zeae*. Approximately × 800. A. Penetration of a cell adjoining a bulliform cell of Rustler (field corn) killed 8 days after inoculation with chlamydospores in water suspension. Transverse section. B. Penetration near a trichome. Pickaninny (sweet corn) killed 6 days after inoculation with chlamydospores in water suspension. Transverse section. C. Penetration of a large thin-walled cell by a hypha that is apparently haploid. S-111 killed 12 days after inoculation with a smut-producing combination of monosporidial lines. Transverse section. D. A 3-dimensional view of the necrotic effect of smut hyphae on Golden Bantam corn. Penetration occurred at *p*. Killed 6 days after inoculation with a smut-producing combination of monosporidial lines. A composite of 3 transverse sections. E. The bulging, curling, and depression-seeking habit of a germ tube, which has recently affected entrance. S-135 (1930) killed 6 days after inoculation with dry chlamydospores by the syringe method. Longitudinal section. F. The typical curling and swelling of the germ tube. S-135 (1930) killed 6 days after inoculation with dry chlamydospores by the syringe method. Longitudinal section.

hyphae gain entrance to the plant and pass from cell to cell by direct penetration through the cell walls. In figure 1 there are representative views of penetration by sporidial germ tubes. Many cases of strongly curled, bulged, and knotted hyphae with points pressed against a slightly yielding, perhaps too mature, epidermis have been observed (Figs. 1, B, E, and F, and 2, C). The evidence leads to the conclusion reached by Brefeld that only tender, not yet heavily cuticularized epidermis can be penetrated. The curling and bulging of the hyphae at penetration are strong evidence that the process is mechanical, and the holes through which the infecting hyphae have passed are certainly small (Figs. 1, A, E, and F, and 2, A and C), although it is perhaps to be expected that the openings are constricted considerably by the time the results of the process can be clearly seen. The depressions of the surface of the leaf are the most common points of penetration, and any structures, such as trichomes, which offer the hyphae footing (Fig. 1, B and E), seem to aid them in their attempts to gain entrance.

It must be considered at present that the fungus forces its way into the host in search of nourishment. In their growth along the leaf surface the germ tubes favor the troughs between epidermal cells. In the depressions they bulge, flatten, and press close to the epidermis, but, unlike apressoria, in many cases they constrict again, grow farther, coil sharply, and effect entrance below the coil. The appearance of many penetrations is quite certainly altered by the rapid elongation of host cells sufficiently tender to be penetrated, and entrance is a process that can be observed only in stages and once per subject by the methods used. Figure 1 represents different stages as well as entirely different views of the process.

In many cases the germ tubes of *Ustilago zeae* seem to be attracted by host-cell nuclei, early stages of successful entries and cases of unsuccessful penetration frequently being found with germ tubes contacting the cell wall directly opposite the nucleus. Strangely enough, however, the stomata call forth no response from the germ tubes, and the latter frequently pass directly over the openings to penetrate the nearby cells directly. The hyphae seem to be most attracted by the cells surrounding the guard cells and those in the vicinity of trichomes or bulliform cells. In only 3 cases were hyphae observed penetrating into the substomatal cavity through the stomata; and in all of these there is the possibility that the inoculum had been forced through the openings by the syringe method of inoculation.

Figure 2 is representative of penetration directly from germinating chlamydospores. The latter are perhaps more important as inoculum than Brefeld seemed to think. Examination of material fresh from the field early indicated that the chlamydospores resulting from primary infections are important as secondary inoculum, *i.e.*, for infections on later plants, at least. It is true that smut sporidia usually are abundant in the spiral

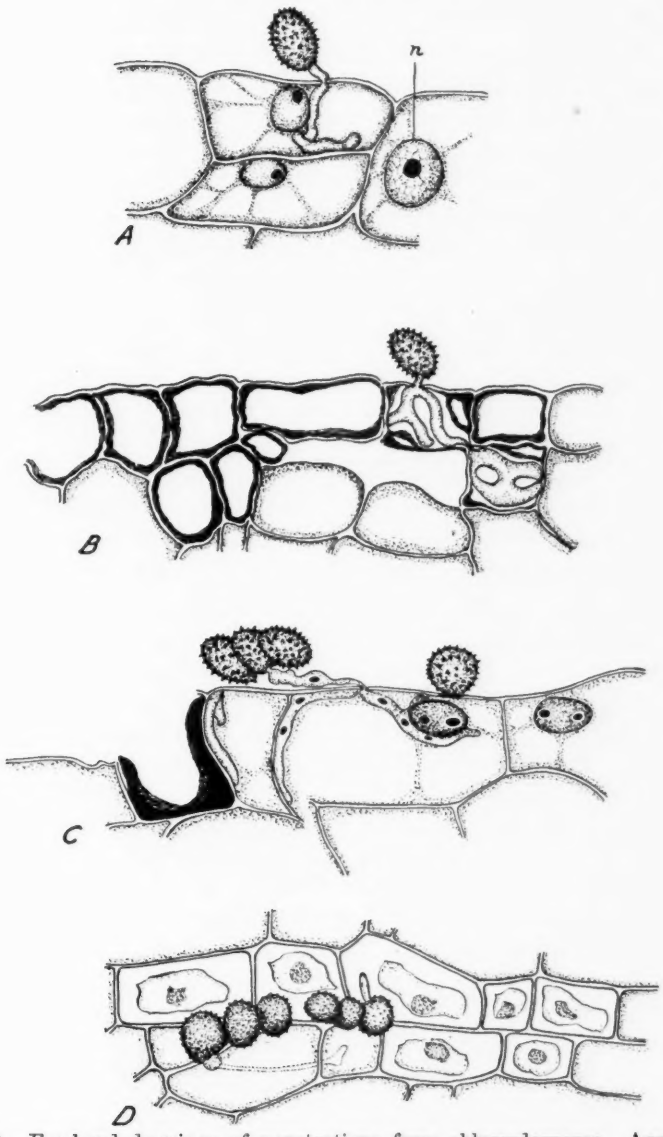


FIG. 2. Freehand drawings of penetrations from chlamydospores. Approximately ×1000. A. Penetration of a cell adjoining a guard cell. S-97 (1930) killed 6 days after inoculation with dry chlamydospores. Longitudinal section stained with Heidenhain's iron-alum haematoxylin. Note the enlarged host nucleus, *n*. B. Penetration and necrosis of cells. Rustler killed 8 days after inoculation with chlamydospores in water suspension. Transverse section. C. Penetration and the tendencies of the hyphae to seek contact with host nuclei. This mycelium is representative of that from chlamydospore germ-tube penetrations and it is possibly diploid. S-97 (1930) killed 6 days after inoculation with dry chlamydospores. D. Surface view of penetration and the necrosis of host cells. S-176 (1930) stained fresh with thionin 48 hours after inoculation with dry chlamydospores.

moisture of corn plants if it is easy to find chlamydospores there; but it also is true that it has required less effort to locate penetrations directly from chlamydospores than from sporidia, both having been injected in water suspension. Should it be the case that reduction division does not take place when a germinating chlamydospore sends out an infecting hypha, one would expect, other things being equal, that infections from chlamydospores would be more successful in producing smut galls than infections from haploid sporidia, because the fusion of complementary lines would then not be necessary for the development of the gall. On the other hand, if the aerial conidia that are liberated from the surfaces of infected tissues (Hanna, 6) are diploid or dikaryotic one should expect them to be equally as effective as chlamydospores. Unfortunately, preparations that throw light on these questions have not been secured.

Brefeld (2), Christensen and Stakman (5), and Platz (7) have called attention to the fact that necrosis is an important symptom of smut infection. Since there seemed to be an association between masses of inoculum and necrotic spots in the material studied histologically, a water extract of chlamydospores (soaked 24 hours) and filtrates from broth suspensions of sporidia were injected into the leaf-spirals of a few plants in the greenhouse. There was no evidence whatever of the necrotic effect of smut on these plants and check inoculations produced marked necrosis; hence it seems fair to consider that the necrosis is due to infection or attempted infection by the smut hyphae. The necrosis of host cells not yet reached by infecting hyphae, as well as that of infected cells, is illustrated in figures 1, D, and 2, B and D.

## DISCUSSION

Although the hyphae of *Ustilago zeae* can penetrate only tender tissue, it is evident, macroscopically as well as microscopically, that they can penetrate and affect tissues which are apparently too mature to produce galls. Meristematic activity and hypertrophy of the host cells are necessary for the formation of the gall or "boil"; and Brefeld's statement (2) that mycelium of *U. zeae* is strictly localized in the host has not yet been qualified or refuted. In view of these facts it seems at present improbable that the mode of entrance of the pathogen can be of great significance in the differential smut reactions of varieties and lines of corn. However, study of a dozen selfed lines and 4 varieties by no means eliminates the posssibilities of entrance through stomata, silks, and other deviations from the seemingly general course of mechanical entrance through tender epidermis.

The necrotic effects of smut inoculum require further study, but there are strong indications that necrosis often prevents the development of smut galls. Christensen and Stakman (4) have reported that different collec-

tions of corn smut resulted in different extents of necrosis, depending upon the line of corn inoculated, and it has been commonly observed at the Minnesota station that different combinations of monosporidial lines cause different amounts of necrosis and different amounts of smut on a given line or variety of corn. At present, it seems most reasonable to assume that the necrosis is perhaps a hypersensitive reaction and depends upon the genotypes of both host and attacking fungus. However, the evaluation of this possible type of smut resistance is, as yet, very difficult for the following reasons:

(1) Only 12 selfed lines of different reactions have been studied histologically, and, although there were generalized differences in the extent of necrosis, in certain cases inversely related to the field reaction of the lines, the inoculum was not limited to one combination of monosporidial lines, one genotype of the fungus;

(2) It is assumed that sexual reproduction (reduction division in chlamydospore germination, plasmogamy and karyogamy in the host) frequently and commonly intervenes in the natural propagation of this highly variable fungus, resulting in an infinite number of genotypes in an organism very easily and widely disseminated by the wind;

(3) No line of corn has as yet proved immune from any given smut complement known to produce galls on other lines, and it seems that this would very likely have occurred if resistance were as independent of host-environment interactions and as purely physiological as a hypersensitive reaction would suggest. Worthwhile results on this question can come only through a strictly controlled study of the reactions of different selfed lines, or $F_1$ generations from homozygous parents, to different smut complements.

## SUMMARY

*Ustilago zeae* enters the corn plant by direct penetration through the epidermis of young cells.

Chlamydospores, as well as sporidia, may send out germ-tubes that penetrate the host directly.

Attention is called to necrosis as a symptom of smut infection and its possible relation to resistance to *U. zeae*.

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# DISTRIBUTION OF DOWNY MILDEW MYCELIUM IN SPINACH FRUITS[1]

L. D. Leach and H. A. Borthwick

(Accepted for publication November 20, 1933)

Mycelium of downy mildew, *Peronospora effusa* (Grev.) Tul. (*P. spinaciae* Laub.) was found in abundance in the calyx tube, integument, and nucellus of spinach fruits, *Spinacia oleracea* L. The favorable nature of this material prompted studies to determine the path of invasion of tissues of the ovule and the possible importance of the presence and distribution of mycelium in relation to seed transmission of the disease.

Either mycelium or oospores of downy mildews in the flower or seed parts of certain truck crops has been observed by Clinton (2), Cook (3) (4), Melhus (7) and Leach (6), while the production of infected seedlings from seed suspected of harboring the fungus has been reported by Angell (1) in tobacco and by Leach (6) in beets.

In April and May, 1930, sporulation of *Peronospora effusa* was observed in abundance on the flowers and fruits of spinach. Material then collected has provided an opportunity to study in some detail the tissues of the spinach fruit invaded by the fungus and the paths traversed during the penetration. Recently, Cook (5) reported his observations on the presence of mycelium of spinach mildew in the "pericarp, funiculus and integuments of the ovule." Our studies, in general, confirm his findings and in addition conclusively demonstrate the presence of mycelium in the nucellus.

Some confusion exists in the literature as to the identification of the various structures of the spinach fruit. The outermost part of a spinach fruit is made up of a thickened calyx tube formed by the fusion of two sepals (Stomps (8)). This tube, at maturity, becomes very stony. Inside of the calyx tube and entirely distinct from it is the very thin membranaceous pericarp. At the stage of development shown in figure 1 the pericarp is a thin membrane composed of a few layers of collapsed cells.

The single, basally attached, campylotropous ovule possesses two integuments. In the early stages of ovule development the 2 integuments are clearly differentiated but, at the stage shown in figure 1, they can be recognized as distinct from each other only in the micropylar region. The seed coat formed by the 2 fused integuments encloses the nucellus, a large part of which persists in the mature seed as a storage tissue known as the perisperm.

[1] Contribution from Divisions of Plant Pathology and Botany, Branch of the College of Agriculture, University of California, Davis, California.

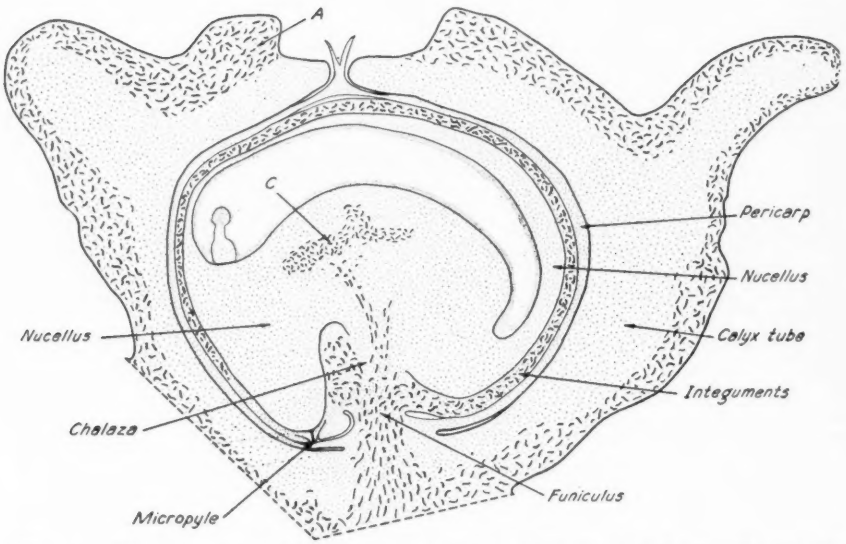


FIG. 1. Diagram of longitudinal section of immature spinach fruit. Distribution of fungus mycelium is shown by irregular broken lines. Letters A and C indicate positions from which sections shown in figure 2, A and C were taken.

The fruits, in which mycelium was found, were collected a few days after fertilization. The embryo at this time had differentiated into a massive multicellular suspensor and globular embryo proper. In the latter there was yet no evidence of the differentiation of cotyledons. Endosperm development at this stage consisted of a single layer of free nuclei lining the embryo sac and enveloping the embryo.

An external examination of the infected plants showed an abundance of conidiophores and conidia on the seed clusters and on localized areas of the leaves, while sporulation or other macroscopic evidence of infection was entirely absent from adjacent areas on the stem. This fact, together with the absence of mycelium from sections of the base of infected clusters, indicates that systemic invasion of seed-bearing branches did not occur. It is probable, therefore, that infection of the fruit occurred through stomata on the calyx tube or pedicel. The mycelium was found in abundance in the outer parts of the calyx tube (Fig. 2, A) while the inner part, which eventually becomes stony, was nearly free of mycelium.

From the base of the calyx tube the mycelium had grown through the funiculus into the integuments (Figs. 1 and 2, D). This, apparently, is the only path of entry to the latter. The inner and outer surfaces of the integuments are covered with definite cutinized membranes that delimit them from the pericarp on the outside and the nucellus on the inside. The fungus mycelium has not been observed to pass through these membranes. Ex-

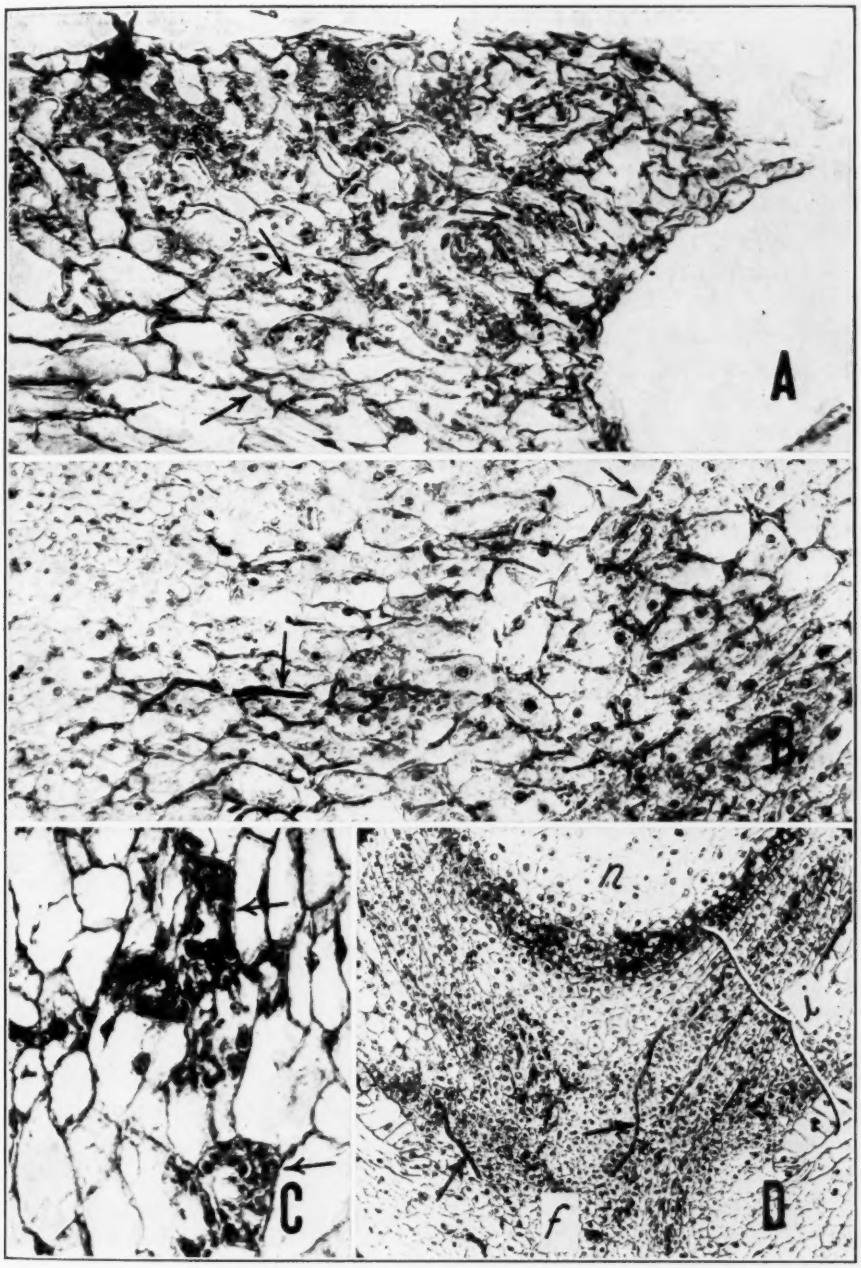


FIG. 2. A. Mycelium in calyx tube. Section from area A, figure 1. ×144. B. Tangential section of ovule showing mycelium in the integuments. ×144. C. Mass of mycelium in nucellus. Section from area C, figure 1. ×307. D. Longitudinal section through base of ovule, cut in a plane at right angles to that of figure 1. Mycelium is shown passing through the short funiculus into the integuments. ×98. *n*, nucellus; *i*, integuments; *f*, funiculus. Arrows indicate location of mycelium.

amination of sections of a number of infected fruits showed that the integuments were uniformly and extensively invaded by mycelium except in the region of the micropyle. A portion of infected seed coat is shown in tangential section in figure 2, B.

The above description represents the extent of invasion found in the majority of infected fruits studied. It is in agreement with the observations of Cook (5), assuming that his so-called "pericarp" is, in reality, the calyx tube. In our studies no mycelium has been found in the membranaceous pericarp, but it seems possible that at certain stages mycelium may gain access.

In one fruit the invasion was not limited to the regions mentioned above but had also involved the nucellus. The mycelium within the funiculus had grown through the chalaza and extended in the form of definitely oriented hyphae upward into the nucellar tissue. Just below the embryo sac these hyphae terminated in a definite area of infected tissue, shown in one plane in figure 1. Examination of the entire series showed that the distribution of the mycelium was limited to a few sections above and below this plane. The hyphae in this area developed into compact masses and appeared to occupy most of the lumen of certain cells (Fig. 2, C). The mycelium was separated from the embryo sac at its closest point by several layers of cells. There was no mycelium in the nucellus in the vicinity of the micropyle or adjacent to the integuments and apparently the only path of entry to the nucellus was through the chalaza.

The distribution of the mycelium in the spinach fruit, as described above, suggested the possibility of seed transmission, and the following studies were made to obtain evidence on this point. Infected flower clusters from the same plants and similar in every way to those used for microscopic studies were tagged and, at maturity, were collected for seed-transmission trials. After being stored through the summer in the laboratory, these seeds were planted in a cool greenhouse in steam-sterilized soil and under isolated conditions. Ten lots of infected seed produced a total of 1260 seedlings, which were kept under observation for 70 days, but no infected seedlings were observed. At the same time 8 lots of seed from infected commercial fields produced 1230 seedlings of which none were infected. Although these trials contributed no positive evidence of seed transmission they were not sufficiently extensive to warrant the conclusion that seed transmission does not occur.

It has been generally observed that seed coats and parts of the fruit wall of onion, beet, and spinach are frequently invaded by the mycelium of certain species of Peronospora. No conclusive evidence exists that such mycelium survives within the seed and infects the embryo at the time of germination. The probability of the survival of mycelium would seem to be

enhanced, however, by its presence, as has been here recorded, within the nucellus of the spinach seed.

SUMMARY

This paper deals with the presence and distribution of the mycelium of downy mildew (*Peronospora effusa* (Grev.) Tul.) in the fruits of spinach (*Spinacia oleracea* L.). Hyphae were found in the calyx tube, funiculus, integument, and nucellus.

The mycelium enters the ovule through the funiculus and from this point invades the integument. The fungus then spreads throughout most of the integument, thus enveloping the ovule. The nucellus is invaded by mycelium that enters through the chalaza.

No evidence of seed transmission was obtained from limited germination trials with infected seed.

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# A NEW SPECIES OF PESTALOTIA ON PODOCARPUS[1]

R. W. G. Dennis, B.Sc.[2]

(Accepted for publication October 21, 1933)

In the Autumn of 1932 a bush of *Podocarpus elongata* growing in the Kibble Palace, Glasgow Botanic Gardens, was observed to be suffering from a severe attack of some form of leaf spot. Microscopic examination showed the lesions to be uniformly associated with the presence of a species of Pestalotia, the identification of which proved a matter of unexpected difficulty. Reference to Guba's Monograph of the genus Pestalotia[3,4] showed it to resemble most closely *P. foedans* Sacc. & Ellis, recorded on the bark of *Chamaecyparis thyoides*. In view, however, of minor divergences in morphological characters and the difference in host genus and organ involved specimens were forwarded to Prof. Guba. After an examination of a pure culture and of diseased leaves, he expressed the opinion that it was an undescribed species.

During the past twelve months the fungus has been kept under observation, and it seems desirable at this stage to put on record such information as has accumulated regarding it.

The leaves of *Pestalotia elongata* may be over 5 inches long but do not normally exceed .25 inch in width. Lesions may develop at any point but are more frequent at or near the apex, where drops of water remain longest unevaporated. When they arise at the tip both sides of the prominent midrib are usually involved, but in the case of lesions originating at other points infection is restricted normally to one side of the midrib. The necrotic areas assume at first a purplish brown tint, which is always retained on the lower side of the leaf. Their upper surface tends to become greyish and even silvery, standing out conspicuously against the dark green of the healthy leaf. A brown line of greater or less width always sharply defines each lesion. (Fig. 1, A.).

Fructifications are produced in abundance on the upper surface of lesions that have attained the greyish stage and are apparent to the naked eye as minute black specks, frequently arranged in rows parallel with the midrib of the leaf. Lesions have not been observed on twigs.

[1] I learn on the authority of Prof. Guba that "The same fungus may be found in the Herbarium of Berlin Botanical Museum under the name of *P. funerea* Desm. *P. Podocarpi* Sacc., nom. nud. The fungus, however, has no relation to *P. funerea.*" Under these circumstances the name *P. Podocarpi* is assigned to it.

[2] I desire to express my indebtedness to Prof. Guba for so freely placing his wide knowledge of this genus at my disposal.

[3] Guba, E. F. Monograph of the genus Pestalotia de Notaris. Part I. Phytopath. **19**: 191–232. 1929.

[4] *Ibid.* Part II. Mycologia **24**: 355–397. 1932.

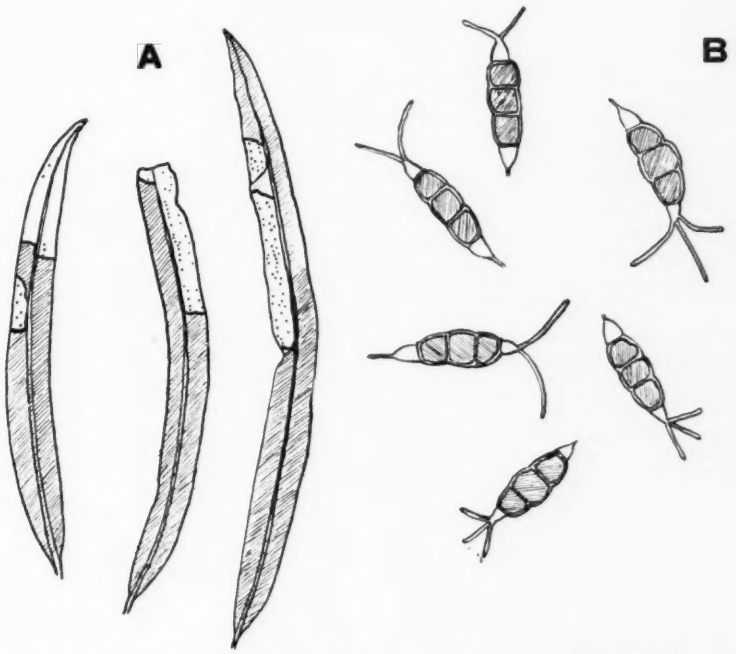


FIG. 1. A. Diseased leaves, showing acervuli. B. Spores of *Pestalotia podocarpi*. × 850.

Owing to the thick, leathery character of Podocarpus leaves, pure cultures are readily obtained from fragments of diseased mesophyll or even more readily by the plating of spore suspensions. On 2 per cent malt agar an even, white, cottony growth quickly results at room temperature. Fructifications are produced in abundance as soon as the plate has become covered with mycelium. They tend to be somewhat larger than those developed on infected leaves and differ further in being well-formed pycnidia, having a definite ostiole from which a black spore coil is extruded. This condition has never been observed on the host, where spore production appears to be far less extensive than in pure culture, probably because of the more rapid exhaustion of the substratum. Spore dimensions, however, remain unchanged. No other form of fructification has been observed.

Cross sections of lesions revealed only mature acervuli. These develop between mesophyll and upper epidermis, the latter being lifted and finally ruptured. The fructification consists of a thin bed of hyphae spread over the upper surface of the mesophyll and a mass of spores filling the cavity. Close examination, however, reveals traces of a hyphal weft completely enclosing the fructification and providing a link with the well-developed pycnidia produced in pure culture. The latter arise as a submerged ball of

tightly interwoven hyphae, which becomes differentiated into an inner thin-walled mass surrounded by a zone 1–3 cells deep of thick-walled, brown hyphae. In the earliest stage observed the formation of a central cavity by the disintegration of the thin-walled, deeply staining tissue has already begun. It is followed by a great increase in volume of the young fructification, the result being a cavity lined and intersected by strands of disorganized material. Considerable tangential stretching is apparent in the cells of the pycnidial wall, from which it appears probable that the increase in bulk is a result rather of high osmotic pressure set up in the disorganized central mass than of rapid growth among the enclosing hyphae. A thick mass of sterile tissue remains at the upper surface of the developing pycnidium, and through this an ostiole is developed by mechanical stretching.

At this stage the uninucleate cells lining the central cavity become elongate and papillate, and form the sporogenous hyphae. In spore production a wall is first laid down delimiting the stalk cell. This is followed by one cutting off the apex. Two more walls then arise dividing the central area of the growing spore into 3 cells, which become brown and thick-walled when growth ceases. The appearance of the apical processes is the last phase in spore development.

Mature spores conform to the general *Pestalotia* type. (Fig. 1, B.). They are spindle-shape, frequently with one side more strongly curved than the other, and with markedly narrow extremities. The setae are simple, neither forked nor knobbed, and 2 or 3 in number. The dimensions quoted in the diagnosis are based on measurements of 100 mature spores from fructifications on foliar lesions.

*Diagnosis:* **Pestalotia Podocarpi,** sp. nov.

Acervuli epiphilli, innati, 200–240 × 130–170 μ atri, in maculis definitis, supra albidis, infra purpureo-brunneis; conidiis 5 cellularibus, anguste fusoideis, utrinque fastigatis, rectis, vel nonnunquam leniter curvatis, vix ad septum constrictis, 17.5–24.5 μ loculis mediis olivaceis vel 2 superioribus leviter obscurioribus, 12–17.5 × 4.4–7.0 μ cellulis extremis turbinatis; ciliis saepe 3 raro 2, conidiophoris brevis.

Pustules epiphyllous, subepidermal, 200–240 × 130–170 μ, black, seated in definite spots, whitish above, purplish brown below.

Conidia 5-celled, narrow fusoid, tapering at both ends, straight or slightly curved, only slightly constricted at the septa, 17.5–24.5 μ. Median cells olivaceous or the upper 2 darker, 12–17.5 × 4.4–7.0 μ, end cells conic, setae usually 3 sometimes 2, pedicel short.

On living leaves of *Podocarpus elongata.*

*Botanic Gardens, Glasgow, Scotland.*

# INFLUENCE OF ROOTSTOCKS ON THE SUSCEPTIBILITY OF SWEET CHERRY TO THE BUCKSKIN DISEASE[1]

T. E. Rawlins and K. G. Parker

(Accepted for publication November 7, 1933)

The symptoms of the buckskin disease of sweet cherry, *Prunus avium* L., were described in earlier publication (1). The most conspicuous symptoms are on the fruits, which are more or less conical in form, shrivel just before maturity, and have short pedicels. During the autumn months a peculiar red coloration is observed on the leaves along the base of the midrib and extending out along the lateral veins. Unlike many graft-infectious diseases the leaves of infected trees show no distinct yellowing or mottling during the growing season.

During an investigation made in 1928 it was observed that trees grafted on Mahaleb, *Prunus mahaleb* L., stock seldom carried fruit that showed symptoms of the disease. In 1929 a careful survey was made of an orchard in which some of the trees were on Mahaleb and some on Mazzard, *Prunus avium* L., stock. It was found that 52 of the 147 trees on Mazzard stock carried diseased fruit, while only 3 of the 82 trees on Mahaleb stock showed diseased fruit. The 3 affected trees on Mahaleb stock were each found to have a large sweet-cherry root growing from above the union, so that these trees were really partially on *P. avium* roots. These roots were cut from 2 of the diseased trees in order to determine whether the trees would recover when only Mahaleb roots remained. These trees have failed to show any evidence of recovery during the 3-year period since the removal of the sweet-cherry roots.

From these observations it appears that the Mahaleb root in some way causes a tree to resist or escape natural infection. On the other hand, even one *Prunus avium* root apparently confers susceptibility upon a tree. Growers in districts where the disease occurs are, therefore, using the Mahaleb stock in all new plantings.

Since very few trees in districts where the disease occurs are on Morello, *Prunus cerasus* L., stock no careful estimate has been made of the percentage of trees on this stock that have become naturally infected. However, such observations as have been made indicate that trees on this stock are very susceptible to the disease and, when infected, exhibit symptoms that are indistinguishable from those shown by diseased trees on Mazzard stock.

[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California.

GRAFTING EXPERIMENTS

Previous experiments (1) have indicated that the buckskin disease may be readily transmitted to healthy trees on Mazzard stock by infected Napoleon scions. More recently an attempt was made to infect young Napoleon trees on Mahaleb stock and others on Morello stock. The method of inoculation was the same as that described in a former paper[2] and consisted in grafting a diseased fruit-bud-bearing Napoleon scion on one branch of a young Napoleon tree and a healthy fruit-bud-bearing Napoleon scion on another branch of the same tree in order to insure fruit production and the possible detection of fruit symptoms. In 2 trees diseased buds were used instead of scions. The disease was found to be readily transmissible to Napoleon trees on Morello stock, 6 of the 7 grafted trees becoming infected. The fruit, leaf, and growth symptoms were similar to those exhibited by young diseased trees on Mazzard stock, namely, a decreased terminal growth, fruit conical in form, having short pedicels and shriveling just before maturity; as with infected trees on Mazzard stock no chlorosis of the leaves was observed.

The trees on Mahaleb stock gave very different results. Of the 8 trees grafted, 7 became very chlorotic and 2 had died within 7 months after grafting. Although 2 of the diseased fruit-bud-bearing scions used as inoculum produced typical diseased fruit, all of the fruit in other portions of the inoculated trees appeared normal. None of the 7 noninoculated check trees on Mahaleb stock showed any evidence of infection.

All of the above experiments have been carried out with young trees from 1 to 3 years old. On March 4, 1933, two large Black Tartarian trees (about 12 years old) having the union with Mahaleb stock at the ground level were each grafted with 10 diseased fruit-bud-bearing Napoleon scions. In one tree the scions were placed on small branches on the south side of the tree and in the other the 10 scions were distributed on small branches throughout the tree. The fruit on both the scions and other portions of the trees appeared to be normal when harvested in May and June, 1933. However, by September, 1933, the leaves on the scions were all extremely chlorotic and those on other portions of the 2 trees exhibited a chlorosis only slightly less severe than that of the scions. Two adjacent check trees of the same age remained normal.

It, therefore, appears that although trees on Mahaleb stock in some way escape or resist natural infection, they become very chlorotic after inoculation by grafting. This has been demonstrated for the Napoleon and Black Tartarian varieties.

[2] Rawlins, T. E., and W. T. Horne, "Buckskin," a destructive graft-infectious disease of the cherry. Phytopath. **21**: 331–335. 1931.

All of the above-described work has been done on trees having the union with the stock near the surface of the ground. The question has arisen as to whether the causal agent of buckskin can pass from an infective Napoleon scion into a Mahaleb seedling and out into a healthy Napoleon scion placed on another branch of the Mahaleb seedling. Accordingly, 14 branched Mahaleb seedlings were planted in an experimental plot and an infected Napoleon scion was grafted on one branch and a healthy fruitbud-bearing Napoleon scion was grafted on another branch of 8 of the seedlings; 3 trees were grafted in 1932 and 5 in 1933. Six check trees were grafted to normal Napoleon scions only, in 1933. The grafting was always done in February or March. All of the diseased scions used as inoculum made little terminal growth, and the leaves on 7 of the 8 became very chlorotic, 2 of the 3 scions set in 1932 died before the second growth season. From the appearance of the 5 diseased scions set February, 1933, it is probable that most of these also will be dead before the 1934 growth season. None of these scions produced fruits.

Of the healthy scions placed on the 8 Mahaleb seedlings inoculated by grafting, all produced leaves which were normal in appearance and produced as much growth as the healthy scions placed on check trees. One of these scions produced 4 fruits which were normal in appearance.

The 6 check trees all remained normal in every respect. Two of the healthy scions placed on the check trees produced fruits that appeared normal. The above evidence, therefore, apparently indicates that the causal agent of buckskin does not readily pass through a Mahaleb stem.

## SUMMARY

Sweet-cherry trees on Mahaleb stock in some way escape or resist natural infection with buckskin. However, trees on this stock become very chlorotic after grafting with diseased Napoleon scions. Such chlorotic trees rarely exhibit fruit symptoms, as contrasted to infected trees on Mazzard and Morello stocks that show little or no chlorosis and produce conical fruits that shrivel just before maturity and have abnormally short pedicels.

When diseased and normal scions were placed on different branches of Mahaleb seedlings the diseased scions became very chlorotic but the infection has not extended through the Mahaleb trees to the normal scions.

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## PHYTOPATHOLOGICAL NOTES

*A Disease of Banana, Markedly Similar to Bunchy Top, Produced by Celery Virus 1 in U. S. A.*—The discovery that banana (*Musa sapientum*) plants of the Lady Finger variety were susceptible to *Celery virus 1* was made on greenhouse-grown plants at Sanford, Florida. The important known virus trouble of this plant is called bunchy top and has been described by such workers as Magee[1] in Australia and Ocfemia[2] in the Philippine Islands. The disease has been considered by Smith[3] as a rosette type of trouble, inferred to belong to the yellows group of viruses. On certain bananas it produces rosette-like symptoms, and it has not been communicated to healthy plants by juice-inoculation methods.

In examining several thousand banana plants in the Sanford district, 6 were found with symptoms suggesting virus infection. These were destroyed by the owners. Natural occurrence of aphids on banana plants in Florida has never been observed by the writer. Medium-size "heads," or corms, were procured from healthy mats of plants in areas far removed from the places where apparently diseased plants occurred. These were planted in the greenhouse and in insect-proof cages. As soon as two leaves had developed they were subjected to experiment. The virus used in these studies has been described by the writer[4] and was obtained from cuttings of the same infected plant of *Commelina nudiflora,* from which was secured the original virus strain designated as *Celery virus 1.* One group of diseased shoots of Commelina was colonized for 14 days with *Aphis gossypii* and another group with *A. maidis.* From 75 to 150 such aphids were caged on banana leaves for inoculation. Of the 15 Lady Finger banana plants inoculated by *A. gossypii,* 13 became diseased, and both of the plants inoculated by *A. maidis* were infected. The incubation period varied from 21 to 33 days. Of the 7 plants held as controls, 4 were infested with virus-free aphids from healthy Commelina; none became diseased.

Early symptoms of celery-virus attack were usually first noticed on the third leaf appearing after inoculation. This leaf changed very little in size and general growth habit from the normal, though it was chlorotic, slow to unfurl, often drooped, and was severely marked with yellowish areas inter-

[1] Magee, C. J. P. Investigation on the bunchy top disease of the banana. Australian Counc. Sci. and Indust. Res. Bull. 30: 64 pp. 1927.

[2] Ocfemia, G. O. Bunchy-top of abacá or Manila hemp I. A study of the cause of the disease and its method of transmission. Amer. Jour. Bot. 17: 1–18. 1930.

[3] Smith, K. M. Recent advances in the study of plant viruses. 423 pp. Blakiston. Philadelphia. 1933.

[4] Wellman, F. L. Identification of *Celery virus 1,* the cause of southern celery mosaic. Phytopath. 24: 695–725. 1934.

FIG. 1. Symptoms of *Celery virus 1* on Lady Finger banana. A. First leaf showing infection. Note darker stripes. B. Portion of one side of healthy leaf. C. Tip of the 7th diseased leaf from plant. Note stunting, malformation, and chlorotic streaks. D. Portion of one side of diseased leaf. Note light color, presence of necrotic streaks, and chlorotic stripes. E. Diseased plant, showing rosette appearance. F. Healthy plant (middle) and 2 diseased plants infected for 3 months. Note differences in height, leaf stunting, necrosis, malformation, and tendency of leaves to cup.

spersed with greener regions on the leaf blade (Fig. 1, A). Succeeding diseased leaves were tightly rolled (Fig. 1, E and F), and a healthy plant growing alongside produced 4 fully developed leaves, while 2 leaves on the diseased plant were pushing out. Diseased leaves were brittle, easily torn, had chlorotically spotted petioles with malformed fibrovascular bundles, and necrotic spots and streaks often occurred on the leaf lamina and pseudostem. There was distinct reduction of petiole and leaf blade and a consequent stunted rosette-like appearance (Fig. 1, E and F), like the bunchy-top symptoms described by Magee and Ocfemia referred to above.

When plants became diseased leaf sheaths were considerably reduced in rapidity of growth and flexibility. Partial strangulation of the newest leaves in the center of the pseudostem ended in their malformation (Fig. 1, E and C) and occasional splitting of the leaf sheaths. The leaves might be small but malformation did not occur if the pseudostem was slit with a knife. Two of the 9 healthy plants had started to produce fruit when experimentation was completed. Three other plants, when cut open, showed evidences of fruit-bud formation. No fruit buds were observed when 9 of the oldest severely diseased plants were dissected.

When aphids were transferred to the banana plants a few were put onto healthy Commelina, cucumber, and celery plants. These always produced typical symptoms of celery-virus infection. The celery virus was always easily recovered from the latter plants either by expressed juices rubbed on cucumber cotyledons (*cf.* Wellman[5]) or through transference by the use of *Aphis gossypii.* However, both methods were used with diseased bananas and it is curious that at no time was it possible to recover the virus from them. Juices from diseased and healthy banana leaves were highly toxic to celery and cucumber seedlings.

Magee's[6] description of bunchy top was confined mostly to its occurrence on Cavendish banana, *Musa cavendishii,* and 2 plants of this species were grown by the writer. Each was inoculated with 175 viruliferous *Aphis gossypii* and became diseased 30 days after inoculation. A week after unfurling, their leaves were slightly cupped, like those described and figured by Magee and Ocfemia. The leaf-cupping symptom was not strikingly present on Lady Finger plants. Diseased Cavendish plants were not maintained long enough to determine whether a rosetted appearance of the plant would result.—F. L. WELLMAN, Associate Pathologist, Bureau of Plant Industry, Washington, D. C.

[5] Wellman, F. L. *Loc. cit.*

[6] Magee, C. J. P. *Loc. cit.*

*Infection of Zea Mays and Various Other Gramineae by the Celery Virus in Florida.*—Studies of *Celery virus 1*, the cause of southern celery mosaic, have shown that the virus has a wide host range. Properties and description of the virus have been recently published by the writer.[1] Doolittle[2] was the first to find that the virus of celery mosaic occurred on the monocotyledonous host (*Commelina nudiflora*). The writer[3] showed while studying eradication measures for control of celery mosaic that around Sanford, Florida, *C. nudiflora* was undoubtedly one of the main reservoirs of the virus and the question of the susceptibility of other monocotyledonous plants naturally arose.

Plants of maize (*Zea mays*) were infected under greenhouse conditions in Florida by means of the viruliferous aphid, *Aphis gossypii*, from colonies on diseased *Commelina nudiflora* and celery. Hundreds of plants were inoculated and about two-thirds of them became diseased. Seedlings of Teosinté (*Euchlaena mexicana*), sweet-, milo-, and Kafir-sorghums (*Holcus sorghum*), wheat (*Triticum aestivum*), and rye (*Secale cereale*) were also successfully infected with *Celery virus 1*. In greenhouse studies sweet, pop, flint, and dent varieties of maize proved about equally susceptible. Several varieties of maize with typical symptoms of the celery virus attack also were found in abundance in fields in widely separate regions in Florida. Juices were obtained from diseased plants from greenhouse and field material, and they produced typical celery-virus lesions on cucumber cotyledons accompanied by systemic symptoms on cucumber and celery. In other properties the virus was identical with *Celery virus 1*.

Incubation periods of the disease on maize varied from 3 to 20 days. The most rapid and severe infection occurred at temperatures between 70° and 90° F., when 15 to 20 viruliferous aphids were placed on seedlings about 3 to 5 days after emergence above ground. Feeding was at first confined to the new, rapidly expanding, first seedling leaf. Primary lesions about 1 mm. in diameter appeared in 3 days around feeding punctures of viruliferous aphids. About 2 days later the lesions had spread downward along the veins of the seedling leaf (Fig. 1, A). Faint systemic symptoms occurred about 8 days after inoculation. Four days later systemic infection effects were distinct (Fig. 1, B). Striping and stunting were marked after the plants had been diseased from 3 to 4 weeks (Fig. 1, D).

The celery virus causes a stripe disease of maize in Florida very similar

[1] Wellman, F. L. Identification of *Celery virus 1*, the cause of southern celery mosaic. Phytopath. **24**: 695–725. 1934.

[2] Doolittle, S. P. *Commelina nudiflora*, a monocotyledonous host of celery mosaic. (Abst.) Phytopath. **21**: 114–115. 1931.

[3] Wellman, F. L. Celery mosaic control in Florida by eradication of the wild host *Commelina nudiflora*. Science n.s. **76**: 390–391. 1932.

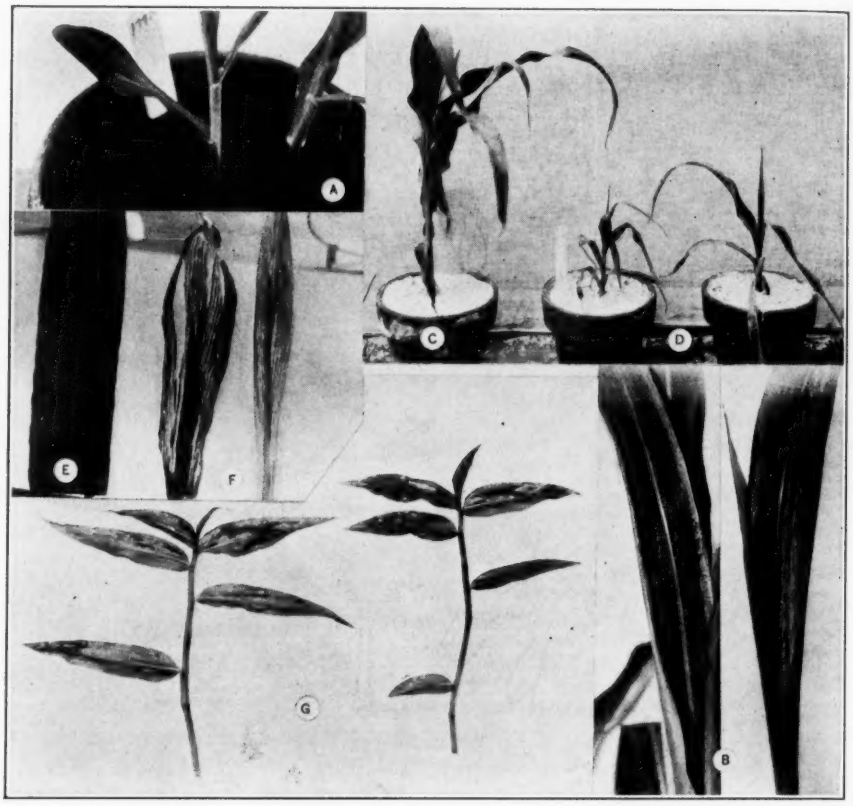


FIG. 1. Symptoms of *Celery virus 1* on maize and *Commelina nudiflora*. A. Primary lesions on first cotyledonary leaf of maize seedlings, 5 days after being infected by viruliferous *Aphis gossypii*. Note spread of lesions downward along veins. B. Early systemic infection symptoms 12 days after infection. C. Healthy maize plant about one-third mature height. D. Maize plants of same age as C but severely diseased with celery virus stripe. E. Portion of leaf from healthy maize plant of same age as leaves in F. F. Celery virus striped leaves from 2 plants infected nearly 6 weeks. Note splitting of lamina, crumpling of tip, and severe stunting. G. Shoots of Commelina severely diseased with *Celery virus 1*. Plants with symptoms of this type were used as the source of virus. Compare these chlorotic patterns with symptom expressions illustrated in B, D, and F.

to but not identical with the white stripe of maize in Cuba. Virus diseases of maize similar to Cuban white stripe described by Stahl[4] are widely distributed in the tropics as is indicated by reports from Hawaii, Africa, Cuba, and Trinidad. The writer visited Cuba recently and found typical symptoms of infection by *Celery virus 1* on celery, squash, cucumber,

[4] Stahl, C. F. Corn stripe disease in Cuba not identical with sugar cane mosaic. Trop. Plant. Res. Foundation. Bull. 7. 1927.

*Commelina nudiflora, C. communis,* pepper, sweet potato, and tomato. Maize, in fields planted at a later time than the vegetables, was diseased with typical white stripe but not with celery virus.

After numerous experiments it was evident that the celery-virus stripe on maize in Florida is transmitted by aphids but not by *Peregrinis maidis,* which Stahl[4] found was the vector of Cuban white stripe. Symptoms on celery-virus diseased plants begin about insect punctures followed by development of round primary lesions that spread downwards (Fig. 1, A). Early evidence of systemic infection consist of numerous light colored, intermittent, elliptical spots of various lengths and widths whose long axes are parallel with veins of the leaves (Fig. 1, B). As infection becomes severe, plants are badly stunted (Fig. 1, D) and are remarkably similar to white stripe-infected plants. They lack, however, the severe rosette and crook-neck symptoms of that disease. Chlorotic patterns often occur similar to cane mosaic on maize (*cf.* Brandes[5]). Occasional plants affected with the celery-virus-stripe have darkened fibrovascular bundles in the stalks similar to Kunkel's[6] description of the Hawaiian virus disease of maize. Well-advanced celery-virus striping of leaves is more translucent than in Cuban white stripe, with distinct demarcation between chlorotic and green areas. There is a tendency to leaf splitting (Fig. 1, F) and sometimes crumpling of leaf tips. Moiré mottling is found on the first systemically infected leaves, and buff colored and necrotic areas on old severely affected leaves.—F. L. WELLMAN, Associate Pathologist, Bureau of Plant Industry, Washington, D. C.

*The Production of Perithecia in Ceratostomella ips Rumb.*[1]—In the course of a study of the interrelation of bark beetles and blue-staining fungi[2] a number of single-ascopore cultures of *Ceratostomella ips* Rumb. were isolated. It was observed that some of the cultures produced both perithecia and conidia, while others produced conidia only. This led to further experiments. The results obtained so far are so different from those reported by other investigators working with other species of Ceratostomella that it seems desirable to place them on record.

Dade[3], in 1928, was the first to demonstrate heterothallism in the genus

[5] Brandes, E. W. Mosaic disease of corn. Jour. Agr. Res. 19: 517–522. 1920.

[6] Kunkel, L. O. A possible causative agent for the mosaic disease of corn. Bull. Exp. Sta. Hawaiian Sugar Plant. Assoc. Bot. Ser. 3: 44–58. 1921.

[1] Paper No. 1265 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Leach, J. G., L. W. Orr, and Clyde Christensen. The interrelation of insects and fungi in the deterioration of felled timber: I. Bark beetles and blue-staining fungi in Norway pine. (In press.)

[3] Dade, H. A. *Ceratostomella paradox,* the perfect stage of *Thielaviopsis paradoxa* (de Seyne) von Höhnel. Trans. Brit. Mycol. Soc. 13: 184–194. 1928.

Ceratostomella. He demonstrated the existence of "+ and −" strains in *C. paradoxa* (de Seynes) Dade. In 1932 Gregor[4] showed that *C. pluriannulata* Hedg. is heterothallic. In the same year Mittmann[5] reported evidence showing that *C. fimbriata* (E. and H.) Elliot and *C. quercus* Georg. are homothallic and that *C. coerulea* Münch. is heterothallic.

Buisman[6] has shown recently that single-ascospore cultures of *Ceratostomella fagi* Loos would produce perithecia, but that cultures from single conidia failed to produce them. However, when 8 single-conidium cultures were mated in the usual manner 2 sexually different groups were discovered, each of which produced perithecia when mated with a culture of the opposite sex. This phenomenon was explained by assuming that segregation for sex was delayed until after the germination of the ascopores and was complete only when the conidia were formed.

In the work here reported the single spores were picked up with a Chamber's micromanipulator and allowed to germinate under observation on a hanging drop of agar after which they were transferred to individual tubes of agar for further observation. Figure 1 shows in diagram the pedigree of the cultures used in the study of perithecial production in *Ceratostomella ips*. It will be noted that of a total of 31 single-ascospore cultures 20 produced perithecia and 11 produced conidia only. From a total of 32 single-conidium cultures from perithecia-producing colonies only 10 produced perithecia. None of the 18 single-conidium cultures, from cultures producing only conidia, formed perithecia. These results cannot be interpreted to indicate heterothallism with delayed segregation for sex, because, when the apparently haploid cultures were mated with each other (See figure 1 for cultures mated), no perithecia were produced. In all cases the fungi were grown on 1 per cent malt-extract agar and control cultures of perithecia-forming strains were included in order to be sure that the medium was suitable for perithecial production. In several cases duplicate cultures were made on other media, but the results were always the same.

As a further check 3 groups of cultures were grown together in Petri dishes on 4.5 per cent malt-extract agar, being planted around the periphery of the dishes as follows: Dish 1—a, 1, b, 2, c, 3; Dish 2—k, 6, l, 7, d, 5; Dish 3—3, 10, f, 11, g, 9; but no perithecia were produced.

In addition, many of the cultures, singly or in combination with others,

[4] Gregor, Mary J. F. A study of heterothallism in *Ceratostomella pluriannulata*, Hedgecock. Ann. Mycol. **30**: 1–9. 1932.

[5] Mittmann, Gertrude. Kulturversuche mit Einsporstämmen und zytologische Untersuchungen in der Gattung *Ceratostomella*. Jahrb. Wiss. Bot. **77**: 185–219. 1932.

[6] Buisman, Christine. Über die Biologie und den Parasitismus der Gattung *Ceratostomella* Sacc. Phytopath. Zeitschr. **6**: 429–440. 1933.

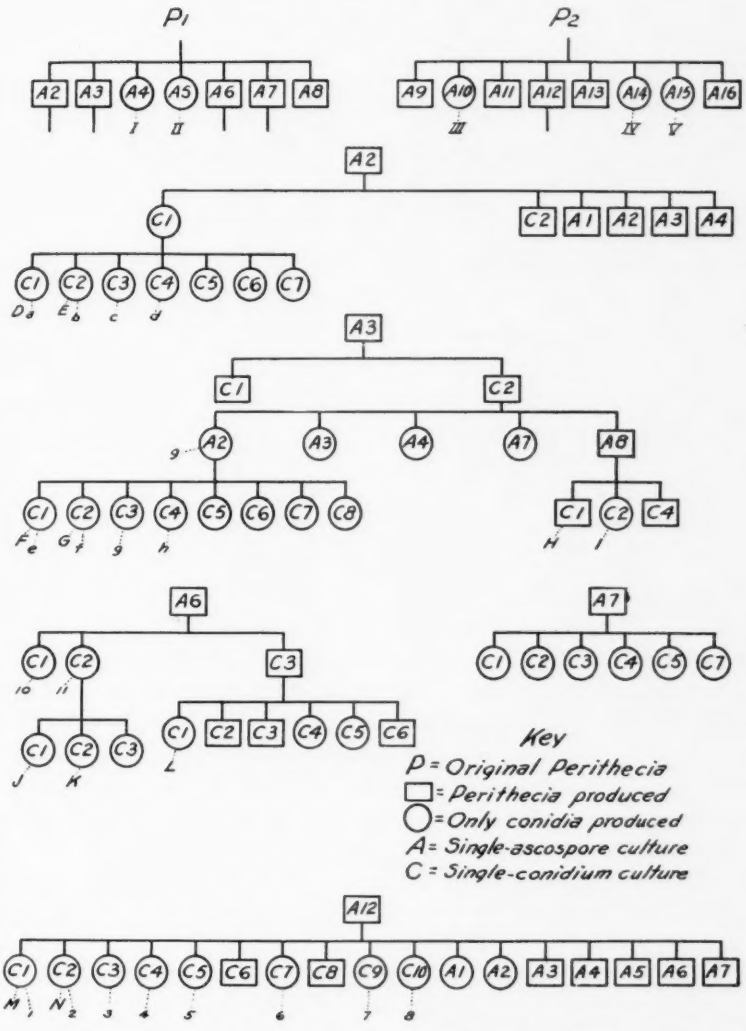
P1
P2
A2 A3 A4 A5 A6 A7 A8
I II
A9 A10 A11 A12 A13 A14 A15 A16
III IV V
A2
C1 C2 A1 A2 A3 A4
C1 C2 C3 C4 C5 C6 C7
D a E b c d
A3
C1 C2
g A2 A3 A4 A7 A8
C1 C2 C3 C4 C5 C6 C7 C8
F e G f g h
C1 C2 C4
H I
A6 A7
C1 C2 C3 C1 C2 C3 C4 C5 C6 C7
10 11
C1 C2 C3 C1 C2 C3 C4 C5 C6
J K L
Key
P = Original Perithecia
= Perithecia produced
= Only conidia produced
A = Single-ascospore culture
C = Single-conidium culture
A12
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 A1 A2 A3 A4 A5 A6 A7
M 1 N 2 3 4 5 6 7 8

FIG. 1. Diagram showing the pedigree of single-spore cultures of *Ceratostomella ips* and indicating the kinds of spores formed by each culture. Four different groups of cultures were mated in all possible combinations within each group. These groups are labelled as follows: Group 1, I to V; Group 2, *a* to *h*; Group 3, 1 to 8; and Group 4, *D* to *N*.

were subjected to ultraviolet light of various intensities and durations without stimulating the formation of perithecia.

From the results obtained by mating, it would appear that the loss of the ability to produce perithecia was due to something other than simple segregation for sex. Of course, it is entirely possible that, if a sufficiently

large number of cultures were mated, the proper combination would be found, but, from the experience of various investigators with heterothallic fungi, it would seem that the matings reported here would have been sufficient to bring together at least some of the complementary sex groups.—J. G. LEACH, University Farm, St. Paul, Minn.

*Types of Mosaic on Sugar Cane in Louisiana.*[1]—Many variations and types of sugar-cane mosaic symptoms have been described and illustrated in the literature. To the writer's knowledge proof has been lacking that such represented other than differences ascribable to varietal reaction or environmental conditions influencing the manifestations of a single causative agent. However, Yoder[2] and Tims and Edgerton[3] have noted unusual behavior of the disease that suggested a difference in cause, although definite separation of strains was not reported. In 1932, the writer found two widely divergent types of mosaic symptoms, (1) mild and (2) severe, occurring naturally on adjacent stools of a single seedling variety, C. P. 28/60, in the nursery plantings of the United States Department of Agriculture at Houma, La. In 1933, 2 additional types (Nos. 3 and 4) were collected in commercial fields of Co. 281. All 4 types have persisted without apparent change through successive vegetative propagations of the infected plants.

In order to determine whether these 4 types represent merely differences in host expression or actual differences in the causative agent, or agents, 4 inoculation experiments employing the needle-prick method were conducted in an insect-proof greenhouse. Usually a high percentage of disease transmission was obtained. Parallel controls, without exception, remained symptom-free. The results may be briefly summarized as follows: The 4 distinct mosaic patterns were readily perpetuated on the original differential varieties (C. P. 28/60 and Co. 281) by transfer of inoculum from diseased to healthy plants of the same variety. All types were then inoculated separately into parallel groups of healthy plants of Co. 281, Louisiana Purple, and the P. O. J. numbers 36-M, 213, and 234. On these varieties types 1, 2, and 4 were not differentiated but gave only indistinguishable mosaic symptoms that were typical of the disease as it occurs on each variety in commercial fields. Type 3, however, reproduced on all these varieties its distinctive yellowish-white stripes that had not previously been encountered except on occasional collections of Co. 281. Inoculum extracted separately

[1] The writer is indebted to Dr. R. D. Rands for valuable advice during the progress of this study and for making the photograph reproduced in figure 1.

[2] Yoder, P. A. Rare cases of mosaic disease in highly resistant varieties of sugar cane. U. S. Dept. Agr. Dept. Circ. 392. 1926.

[3] Tims, E. C., and C. W. Edgerton. Behavior of mosaic in certain sugar-cane varieties in Louisiana. (Abst.) Phytopath. 22: 27. 1932.

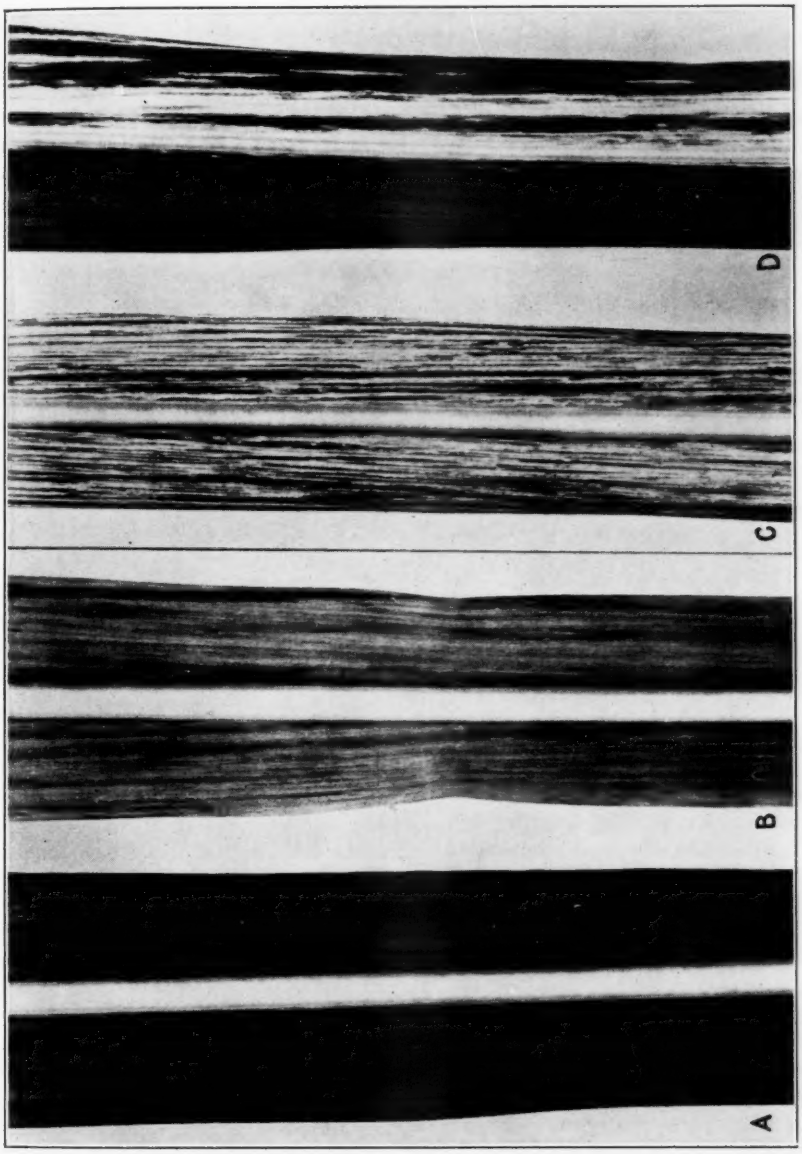


FIG. 1. Three types of mosaic symptoms on leaves of the sugar cane variety C. P. 28/60. A. Healthy; B. Mosaic type No. 1. C. Type No. 2. D. Type No. 3.

from the 3 groups of Louisiana Purple (and later other varieties) showing identical symptoms as well as from the type-3 plants and introduced into healthy groups of C. P. 28/60 and Louisiana Purple invariably reproduced in typical pattern types 1, 2, and 3 on C. P. 28/60 (Fig. 1). Type 4, while indistinguishable from type 3 on this variety, invariably produced the ordinary pattern on Louisiana Purple in contrast with the severe symptoms of type 3. Preliminary tests with inoculum mixtures failed to reproduce type 3 from any combination of the remaining types.

From the consistency with which the 4 types of symptoms have been reproduced in these experiments, a difference in causative agent, or agents, would seem to be indicated as nearly as can be predicted on symptomatology. Possibly these types are the expression of individual strains or temporary modifications of a single virus, or they may represent distinct virus diseases. In either case it is recognized that they may be further resolved by the discovery of additional differentiating hosts. The types may be tentatively described as they occur on 1 to 4-months-old plants, as follows:

*Type 1* is characterized by a slight mottling with very little chlorosis and no noticeable stunting of C. P. 28/60 (Fig. 1, B) and by the production of ordinary (typical for the variety) mosaic symptoms on Louisiana Purple, Co. 281, P. O. J. 36-M, P. O. J. 213, and P. O. J. 234.

*Type 2* appears as a severe mottling with large chlorotic areas, a varying extent of necrosis, and marked stunting of C. P. 28/60 (Fig. 1, C) and only ordinary mosaic symptoms as in type 1 on the other varieties.

*Type 3* first becomes evident on C. P. 28/60 by the development of elongated, almost white blotches or islands, some of which later coalesce into long, yellowish white streaks, often running the full length of the older leaves (Fig. 1, D). The streaks may follow the midrib, and are frequently accompanied by necrosis, sometimes so severe as to produce temporary blighting, and sometimes death, of the growing point. Severe symptoms similar to the above appear also on Louisiana Purple and the four other varieties.

*Type 4* on C. P. 28/60 is identical in appearance with type 3, but in common with types 1 and 2, manifests only ordinary mosaic symptoms on the five other varieties.

Thus far, type 1 has been obtained only from 4 C. P. seedlings; types 2 and 4 quite frequently from Co. 281, and type 4 from all specimens of P. O. J. 213 thus far collected and tested. Type 3 is apparently very limited in distribution. However, more extensive surveys of commercial varieties and fields, and inoculation tests now in progress should throw more light on type distribution, varietal susceptibility, and economic importance as well as the phenomenon of recovery on which many conflicting (unpublished) data have been accumulated.—EATON M. SUMMERS, Bureau of Plant Industry, Houma, Louisiana.

*Panicles of Rexoro Rice Injured at Emergence by Sun Scald.*—During the latter part of August, 1932, a characteristic blighting of panicles of Rexoro rice in some fields south of Lake Charles, Louisiana, was called to the attention of the senior author by C. C. Collett, the local county agent. In a certain field 5 to 10 per cent of the plants scattered throughout the area were severely injured, and the farmers in the vicinity feared that some new disease was attacking their rice. This severely attacked field was visited again on September 7 and 13. The blighting occurred to some extent over this entire period of about three weeks.

It was distinctly different from "straighthead" in that the affected panicles or portions of panicles were bleached and killed as if scalded. In some cases only the upper portions of certain spikelets showed injury, and the lower portions of the spikelets and panicles apparently had been protected, when the injury occurred, by the upper parts of the leaf sheath. Some panicles showed injury only in the upper or middle portions, while others were completely blighted. The badly blighted panicles remained erect, as in straighthead, while the uninjured panicles turned down as they began to fill.

The most recently blighted spikelets were noted on panicles that had emerged within one or two days. These spikelets still retained some green color, those that had been blighted for a slightly longer time were bleached nearly white, and those that had been blighted for a still longer time were overgrown to a greater or lesser extent by saprophytic black molds. On the basis of the relative abundance of the black molds, particularly at the time of the last visit, it was possible to estimate the approximate time when the various panicles had been injured.

Through the courtesy of Mr. Collett, the weather records for Lake Charles for 1932 and the preceding 3 years were secured. It was found that during the 3-week period in 1932, in which blighting of Rexoro occurred, higher temperatures and greater wind velocities had been recorded than in the same period of the preceding 3 years. Undoubtedly these high temperatures and wind velocities, together with bright sunshine, produced the scald injury. The panicles of Rexoro appear to be very delicate as they emerge from the boot and, therefore, seem especially susceptible to this type of injury. J. Mitchell Jenkins states that once or twice sun scald has been observed on Rexoro panicles at the Rice Experiment Station, Crowley, La.

A somewhat similar injury was reported by Tisdale and Jenkins[1], both for California and Louisiana, on varieties other than Rexoro. However, the injury in California apparently was somewhat different, as it was "more noticeable on levees and on land where there is a lack of water,"

[1] Tisdale, W. H. and J. M. Jenkins. Straighthead of rice and its control. U. S. Dept. Agr. Farmers' Bull. 1212. 1921.

whereas the injury to Rexoro occurred on plants scattered throughout normally submerged portions of the field and apparently was no more abundant on the levees than elsewhere.

After September 13 no further injury was noted by either Mr. Collett or the farmer in the field of Rexoro under observation. While some reduction in yield resulted, a fairly satisfactory crop was obtained.—E. C. TULLIS, A. L. SMITH, AND A. G. JOHNSON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

*The Inability of Aplanobacter Insidiosum to Enter Alfalfa Seedlings in the Absence of Wounds.*[1]—It has been generally accepted that the entrance of the wilt organism, *Aplanobacter insidiosum* L. McC., into alfalfa is effected only through freshly made wounds of any type or location, on either the above- or below-ground plant parts. To determine whether the bacteria are capable of entering any natural portal the following experiment was undertaken in the fall of 1932. A greenhouse bench, $40 \times 3$ ft., was so filled with composted sod soil that from the center to either end of the bench there was a drop of about 4 inches in the soil level. The bench was divided into 20 blocks $2 \times 3$ ft. in area.

Eight hundred one-month-old seedlings of Grimm alfalfa were inoculated by severing the ends of the roots under a bacterial suspension and planted in the 4 center blocks at the crest of the soil slope. Each successive block to either end of the bench was then planted to noninoculated seedlings at the rate of 200, 150, 120, 100, 80, 60, 40, and 32 plants. Approximately 2500 plants were employed.

After 5 weeks, when symptoms were first noted in the inoculated area, all the watering for the bench was done from the center blocks, the slope of the soil being sufficient to carry the water rapidly to either end of the bench. As the plants began to die off in the infected area, the force of the water was strong enough to distribute the dead plant parts over the entire bench. The top growth was cut once during the course of the experiment with due precautions to avoid any infection of the noninoculated plants. Water was kept away from the plants until the wounds made by cutting the tops were closed.

At the end of 5 months a detailed examination of all individuals was made for evidences of wilt infection. Of the 800 plants inoculated only 5 per cent were found healthy and 20 per cent were slightly infected, whereas the remainder were moderately to severely diseased or dead. In no instance was even a slightly infected plant found in any other part of the bench, irrespective of the rate of planting. It can be concluded that the bacteria, at least under the conditions of the above experiment, are unable to enter

[1] Journal Series Paper No. 151, of the Nebraska Agricultural Experiment Station.

alfalfa plants in the absence of freshly made wounds. It may also be safe to assume that the organism causing bacterial wilt is unable to enter any natural portal on older alfalfa plants under ordinary field conditions.—GEORGE L. PELTIER, Nebraska Agricultural Experiment Station, Lincoln, Nebr.

*The Effect of Mildew Infection on the Response of Wheat-Leaf Tissues Normally Resistant to Leaf Rust.*[1]—The effect of infection by one fungus upon the response of the host plant of another fungus occasionally has been mentioned in phytopathological literature. A striking demonstration of the breaking down of the resistance to leaf rust of the leaf tissues of certain wheat varieties by earlier infection by mildew has been observed repeatedly at Manhattan, Kansas, in greenhouse experiments with *Puccinia triticina* Eriks. The phenomenon in these cases has consisted in the development of localized areas of susceptible tissue in leaves, normally resistant to leaf rust, after they had been attacked by mildew.

Early in the leaf-rust studies the writer noted that occasional large, normal uredia developed on wheat plants resistant to the physiologic form with which they were inoculated[2]. These were at first regarded as chance mixtures of some other form, but reisolation cultures proved this not to be the case. Such cultures, when used to inoculate seedlings of the variety upon which the uredia had developed, produced only the typical resistant type of reaction. Tests with differential varieties proved them to be pure cultures of the form originally used as inoculum.

A closer examination of many such uredia revealed that they nearly always were closely associated with infections of mildew, *Erysiphe graminis* DC. Uredia developed embedded directly in the mycelial weft of the mildew, in the narrow chlorotic area immediately surrounding the mycelial weft, or in the chlorotic area on the surface of the leaf opposite a mildew spot.

The effect of mildew infection on the response to leaf rust of a plant of the variety Warden (C.I. 4994) is shown in figure 1. This plant was grown in the greenhouse in 1931 and was inoculated with a pure culture of leaf rust form 9. The mildew developed from natural infections. It will be noted that several large uredia have developed in the mildewed areas, while outside of such areas leaf-rust infection is manifest only as

[1] Contribution No. 341 from the Department of Botany, Kansas Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, cooperating.

[2] EDITOR'S NOTE: Following the acceptance of the manuscript for this note it was discovered that the same phenomenon is discussed in a manuscript by E. B. Mains, submitted for publication in PHYTOPATHOLOGY on Jan. 10 and accepted on Jan. 17, 1934.

FIG. 1. Sections of a leaf of Warden (C. I. 4994) spring wheat affected by mildew and leaf rust. A. Lower surface showing uredia of leaf rust in the mycelial wefts of mildew. B. Upper surface of the same leaf showing leaf-rust uredia in chlorotic areas on the leaf surface opposite a mildew spot. Small chlorotic flecks caused by leaf-rust infection in mildew-free tissue appear in both A and B.

numerous sterile flecks. A section of the lower surface of the leaf with uredia imbedded in several of the mildew mats is shown at A, while B shows a section of the upper surface of the same leaf with uredia in a chlorotic spot resulting from mildew on the opposite surface.

Warden is consistently highly resistant to physiologic form 9, with which this plant was inoculated. Flecking, but no uredia, usually result from such inoculation. Isolations from several uredia on this plant gave only pure form 9, which in turn produced only flecking on mildew-free seedlings of Warden.

No explanation is available for the changing by mildew of localized areas of leaf tissue from a resistant to a susceptible condition with regard to leaf rust. Since spots affected by mildew usually are accompanied by chlorosis, it would seem obvious that some change has occurred in the leaf tissues. The fact that leaf rust develops normally on such tissues suggests that these changes are of a chemical nature. It seems probable that the mildew fungus splits some complex compounds, which the rust fungus is unable to use, into simpler compounds upon which it is able to subsist.—C. O. JOHNSTON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Kansas Agricultural Experiment Station.

*The Occurrence of Stem Rot of Rice in California.*—The rice crop in California has been exceptionally free of disease. Therefore, finding stem rot (*Leptosphaeria salvinii* Catt.) there in 1932 was of considerable interest. In the spring of 1932, seed of Early Prolific (known also as Early Blue Rose), obtained from the Woodland Rice Mill, Woodland, Yolo County, Calif., was sown in the replicated varietal plots at the Biggs Rice Field Station. In 1931, this mill had grown a small acreage of Early Prolific in Yolo County from seed obtained from Arkansas that year, and apparently the seed sown at Biggs in 1932 came from that crop.

In examining the rice varieties at the Station in September, 1932, a plant of Early Prolific was found to be infected with stem rot in the conidial and sclerotial stages. No other infected plants were found.

In the fall of 1933 stem rot was observed on 6 varieties in the Station plots and on Early Prolific in a commercial field in Yolo County, but apparently it had done no serious damage. It is, therefore, probable that there were other infected plants present in 1932 that were overlooked.

Owing to the general distribution of the disease in Arkansas, it is probable that the conidia and sclerotia of this fungus are carried on seed rice. Since stem rot was first found in California on an Early Prolific plant, it seems very likely that the disease was introduced with seed of that variety in 1931.—E. C. TULLIS, JENKIN W. JONES, AND L. L. DAVIS, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the California Agricultural Experiment Station.

## *THE CROP PROTECTION INSTITUTE*

SUMMARY OF PROGRESS

January 1, 1934

The following is a condensed summary of the progress and work of the Crop Protection Institute from the time of its organization, 12 years ago, to the first of the current year. It has been prepared in order to give a general view of the institute's operations and of the results accomplished and to summarize briefly the method that it follows in all of its research projects.

From the beginning all of the institute's activities have been controlled by a Board of Governors of 9 men. Three members of this board are chosen by The American Phytopathological Society, one being named each year for a 3-year term. Three members are chosen similarly by the American Association of Economic Entomologists. Two are named by the Association of Official Agricultural Chemists. One is designated by the National Research Council.

The board elects its own chairman who serves as administrative officer in financial matters, and is *ex officio* a member of the institute's various technical committees in order to perform necessary administrative functions. Technical direction of the research projects, however, lies with technical committees representing the board. The board elects a secretary-treasurer who for many years has been Mr. Paul Moore, who has his office in the building of the National Research Council, in Washington. The financial depository of the institute also is in Washington.

Recently, at the suggestion of the chairman of the board, provision was made for election of a vice-chairman, who would work with the chairman and help him in various contacts with industrial organizations.

Under the institute's invariable procedure every research project or development undertaking is maintained as a separate entity. It has its own technical committee who plan the project in detail, review the progress of it, select the investigator or investigators employed on the project, and examine and interpret the results.

Initial contacts with various industries that have possible research to undertake are made by the chairman of the board with the help of the vice chairman. These contacts are necessarily many and varied. Some of them are made because inquiries are addressed to the institute. This, of course, occurs to an increasing degree as the work of the institute becomes more and more wide-known. Other contacts are made because the general nature of a given industry, or the nature of products made by it, suggest that

interesting research could be undertaken. This is especially true in the chemical industry, where invariably there are various by-products, some of which have never been explored as to their application to horticulture, agriculture, animal industry, or other related activities. Such contacts as these have been made with several hundred representatives of the industrial world. Out of these has come the institute's research program during the past 12 years.

If a contact develops the possibility of a research project, the matter is immediately submitted to proper technical experts on the board of governors and frequently to additional experts from among the scientific membership of the institute. Through such consultation the initial study of a possible project is made, tentative plans are drawn up, and a tentative budget is worked out. If the entire matter looks sound and sufficiently interesting scientifically, it is submitted in time to the board of governors of the institute as a proposed project, and the board votes whether the institute shall or shall not enter into an agreement to carry on the projected research.

If a decision is made to enter upon the new project, a technical committee is named immediately and this committee, as already indicated, represents the board in handling all details of the project, including the selection of a man, or men, to be employed and the layout of the research program. Under the institute's practice the committee, although fully responsible for the propect, works in close harmony with the technical staff of the company that is providing funds for the new project. Through this close cooperation results are secured that would not otherwise be possible, while at the same time the institute retains full authority and responsibility and thus safeguards the scientific status of its work.

The following is a brief summary of research projects undertaken to date. Within the space available only the title of a project and brief facts as to research men employed on it and the resultant publications can here be given. Full technical reports on many of these research projects will be found in the institute's bulletin series.

*Sulphur Investigations.*—Supported jointly by the Freeport Sulphur Company, the Texas Gulf Sulphur Company and the Union Sulphur Company. Headquarters: the Missouri Botanical Garden, the Geneva, N. Y., Agricultural Experiment Station, and the Boyce Thompson Institute. Investigators, H. C. Young, A. Hartzell, F. H. Lathrop, L. Tisdale. Five publications, Bulletins Nos. 3, 5, 6, 7, and 8.

*Scalecide Investigations.*—Supported by the B. G. Pratt Company. Headquarters: Pennsylvania Agricultural Experiment Station, State College, Pa. Investigator, John W. Miller.

*Furfural Investigations.*—Supported by the Quaker Oats Company. Head-

quarters: Iowa State College, Ames, Iowa. Investigators, H. H. Flor and C. S. Reddy. Publications, Bulletins Nos. 13 and 24.

*Crown Gall Project.*—Supported by U. S. Department of Agriculture, American Association of Nurserymen, Iowa State College, University of Wisconsin, and Boyce Thompson Institute. Headquarters: Iowa State College, Boyce Thompson Institute, and University of Wisconsin. Investigators, M. K. Patel, A. J. Riker, J. H. Muncie, and W. B. Shippy. Twelve publications, Bulletins Nos. 9, 11, 16, 18, 19, 20, 21, 25, 26, 27, 29, and 39.

*Copper Studies.*—Supported jointly by Nichols Copper Company and Goldsmith Bros. Smelting and Refining Company. Headquarters: Boyce Thompson Institute. Investigator, Frank Wilcoxon.

*Standard Oil Emulsion Project.*—Supported by the Standard Oil Co. (Indiana). Headquarters: the Illinois Agricultural Experiment Station, Urbana, Ill. Publications, Bulletin No. 33.

*Flit Investigation.*—Supported by the Standard Oil Company of New Jersey. Headquarters: New Jersey Agricultural Experiment Station. Investigator, F. C. Nelson. Publication, Bulletin No. 12.

*Kip and Bovinol Project.*—Supported by Standard Oil Co. (Indiana). Headquarters: Purdue University Agricultural Experiment Station. Investigator, H. G. Butler.

*Bayer Company Project.*—Supported by Bayer Company, Inc. Headquarters: the Boyce Thompson Institute. Investigator, J. L. Horsfall. Publication, Bulletin No. 15.

*Thallium and Mercury Project.*—Supported by Bayer Company, Inc. Headquarters: Boyce Thompson Institute. Investigator, C. R. Orton.

*Airfloat Project.*—Supported by Standard Oil Company of New Jersey. Headquarters: New Jersey Agricultural Experiment Station. Investigator, R. C. Burdette.

*Horticultural Sprays Project.*—Supported by Standard Oil Company of New Jersey. Headquarters: Connecticut (State) Agricultural Experiment Station. Investigator, Neely Turner. Publication, Bulletin No. 38.

*Pyrethrum Project.*—Supported by Standard Oil Company of New Jersey. Headquarters: New Jersey Agricultural Experiment Station; Lexington, Kentucky; Florida Experiment Station; and Pennsylvania. Investigator, R. E. Culbertson.

*Phinotas Investigation.*—Supported by Phinotas Chemical Company. Headquarters: New Hampshire Agricultural Experiment Station. Investigator, M. Stewart. Publication, Bulletin No. 14.

*Flotation Sulphur Project.*—Supported by Koppers Products Company. Headquarters: University of Illinois. Investigators, M. A. Smith, J. M. Hamilton, O. H. Richter, and C. E. Baker. Publications, Bulletins Nos. 28 and 33.

*Oxidized Oils Project.*—Supported by Kay Laboratories, Inc. Headquarters: Maryland Agricultural Experiment Station. Investigator, J. L. Hoerner. Publication, Bulletin No. 23.

*Shale Oils Project.*—Supported by Tennessee Copper & Chemical Company. Headquarters: Ohio Agricultural Experiment Station and at Uvalde, Texas. Investigators, R. Melvin, H. F. Dietz, G. S. Langford.

*Ansect Project.*—Supported by Interstate Chemical Company. Headquarters: Maryland Agricultural Experiment Station. Investigator, L. L. Williams.

*Organic Chemicals Investigations.*—Supported by Monsanto Chemical Company. Headquarters: Illinois Agricultural Experiment Station. Investigator, G. L. Hockenyos. Publication, Bulletin No. 31.

*Pyrethrum Sprays.*—Supported by J. C. Makepeace. Headquarters: Columbus, Ohio, and Wareham, Mass. Investigators, H. G. Walker, E. A. Richmond, and G. L. Walker.

*Nurserymen's Tape.*—Supported by Johnson & Johnson. Headquarters: University of Wisconsin. Investigator, S. S. Ivanoff.

*Kay Fungicides Project.*—Supported by Kay Laboratories, Inc. Headquarters: Delaware Agricultural Experiment Station. Investigators, D. S. Loveland, and J. S. Trumbower.

*Kerosene Household Sprays Investigations.*—Supported by Deep Rock Oil Corporation. Headquarters: Iowa State College. Investigator, H. H. Richardson. Publication, Bulletins Nos. 34 and 37.

*Cocoanut Oil Emulsion Studies.*—Supported by Research Corporation. Headquarters: Boyce Thompson Institute. Investigator, L. E. Dills.

*Volck Project.*—Supported by California Spray-Chemical Company. Headquarters: Kansas Agricultural Experiment Station, Manhattan, Kansas. Investigators, W. G. Bruce, H. L. Caler, and D. G. Hall. Publications, Bulletins Nos. 17, 22, and 35.

*Control of Fruit Moth by Oil Spray.*—Supported by California Spray-Chemical Corporation. Headquarters: Delaware Agricultural Experiment Station. Publication, Bulletin No. 41. Investigator D. McCreary.

*Codling Moth Studies, Washington.*—Supported by California Spray-Chemical Corporation. Headquarters: Washington Agricultural Experiment Station. Investigators, T. P. Strand, K. M. Gillies, and C. Miller.

*Codling Moth Studies, California.*—Supported by California Spray-Chemical Corporation. Headquarters: University of California, Berkeley. Investigator, Mr. Hensill.

*Oil Residues Project.*—Supported by California Spray-Chemical Corporation. Headquarters: New Hampshire Agricultural Experiment Station. Investigator, L. S. Groves.

*Diamalt Project.*—Supported by Standard Brands, Inc. Headquarters:

Maryland Agricultural Experiment Station. Investigator, W. A. Thorne.

*Thiocide Project.*—Supported by Midwest Chemical Company. Headquarters: University of Illinois, Urbana. Investigators, G. L. Hockenyos and Mr. Blair.

*Organic Insecticides Investigations.*—Supported by Dow Chemical Company. Headquarters: Iowa State College. Investigator, J. F. Kagy.

*Sulphur Project.*—Supported by Mac Sulphur Company. Headquarters: Delaware Agricultural Experiment Station. Investigators, J. C. Carter, Raymond Russell.

*Iodine Project.*—Supported by Iodine Educational Bureau, Inc. Headquarters: New Jersey Agricultural Experiment Station. Investigator, C. H. Blasberg.

*Sharples Solvents Project.*—Supported by Sharples Solvents Corporation. Headquarters: Ohio Agricultural Experiment Station, Wooster, and Philadelphia. Investigator, M. M. Darley.

*Liquid Carbon Dioxide Project.*—Supported by Liquid Carbonic Corporation. Headquarters: Iowa State College and Chicago. Investigator, R. M. Jones. Publications, Bulletins Nos. 40 and 42.

*Copper Fungicides Project.*—Supported by California Spray-Chemical Corporation. Headquarters: New Jersey Agricultural Experiment Station. Investigator, A. C. Sessions.

*Organic Insecticides and Fungicides (Two projects).*—Supported by National Aniline and Chemical Company, Inc. Headquarters: Delaware Agricultural Experiment Station, New Hampshire Agricultural Experiment Station, Florida, and Mississippi. Investigators, G. L. Walker and C. N. Priode.

*Halowax Project.*—Supported by Halowax Corporation. Headquarters: Ohio State University and Sanford, Florida. Investigator, E. P. Breakey. Publication, Bulletin No. 45.

*Copper Fungicides Project.*—Supported by Nichols Copper Company. Headquarters: Delaware Agricultural Experiment Station. Investigator, A. A. Nikitin.

*Copper in Plant Nutrition.*—Supported by Nichols Copper Company. Headquarters: Delaware Agricultural Experiment Station. Investigator, R. Russell. Publication, Bulletin No. 43.

*Repellents.*—Supported by Standard Oil Company of New Jersey. Headquarters: New Jersey Agricultural Experiment Station. Investigator, W. Moore.

*Sulphur Project.*—Supported by Freeport Sulphur Company. Headquarters: Delaware Agricultural Experiment Station. Investigator, P. D. Peterson. Publication, Bulletin No. 44.

*Sulphuric Acid in Weed Control Project.*—Supported by Freeport Sulphur Company. Headquarters: University Farm, Davis, California. Investigator, W. E. Ball.

*Sulphur and Carbon Bisulphide (Two projects).*—Supported by Stauffer Chemical Company. Headquarters: Connecticut Agricultural Experiment Station and Massachusetts Agricultural Experiment Station. Investigators, M. V. Anthony and T. T. Ayers.

*Plant Extracts as Insecticides.*—Supported by S. B. Penick & Company. Headquarters: New Hampshire Agricultural Station. Investigator, O. J. Smith.

*Copper Fungicides Project.*—Supported by Shepherd Chemical Company. Headquarters: New Hampshire Agricultural Experiment Station and Delaware Agricultural Experiment Station.

W. C. O'KANE,
*Chairman, Board of Governors*